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THE FOLLICLE SACS OF THE AMPHIBIAN OVARY.

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In 1738, Swammerdam (6) stated that just after the eggs of the frog have been laid, one can find on the inner wall of the ovary, "some empty and very delicate membranes which had served to invest the eggs that had been already discharged from the ovary. . . . These particles were most beautifully interwoven with blood vessels to which they were fixed as to so many stalks." Swammerdam does not give the origin of these membranes nor does he state how the eggs pass from the ovary into the body cavity.

Over one hundred years later, Thompson (7) wrote that the ovarian egg of the frog and toad "is surrounded by a thin vascular sac formed by the dilation of the ovisacs which hang into the general ovarian cavity. This capsule or ovisac is attached to the rest of the ovarian substance by a broad band rather than by a narrow pedicle; and when the yolk or ovarian ovum is mature, it escapes from the ovisac by the formation of an aperture in the remote or free side of this capsule, somewhat in the same manner as occurs in the calyces of the bird, but with a wider aperture. Through the apertures of the general ovarian capsule the numerous ova pass into the abdominal cavity." In thus stating that the ovarian eggs first fall into the general cavity of the ovary and later pass into the body cavity through openings in the ovarian wall, Thompson is in agreement with earlier writers, Rathke (4) and Lereboullet (3). More recently this view has been opposed by Brandt (1) who examined the outer surface of the ovary of *Rana temporaria* as the eggs were about to pass into the body cavity and found a round hole above each egg through which a larger or smaller part of the egg protruded. This discovery led him to state: "Es ist mithin klar, dass für jedes sich lösende Ei

eine besondere Austrittsöffnung in die Peritonealhöhle hergestellt wird."

According to Brandt, the ovarian egg of *Rana* is surrounded by membranes whose origin he describes as follows: "Die Follikel des Ovariums dürften dadurch entstehen, dass die einzelnen sich vergrössernden Eianlagen von den benachbarten, sich proliferirenden und abplattenden Elementen, sowie von den Bindegewebselementen der Ovarialwandung umwachsen werden." These membranes are said to form the follicle sacs that are found on the inner wall of the ovary after the eggs have passed into the body cavity, but no mention is made of their later history. During his investigations, Brandt carefully injected colored fluids into the ovary just after the eggs had been laid and found that none of the fluid ever came through the walls of the follicle sacs. He was thus able to show conclusively that there is no communication between the cavity of the ovary and the body cavity through the walls of the follicle sacs. The latter have but the one opening which leads outward into the body cavity.

The investigations recorded in the present paper were made to supplement the work of Brandt and others with a more detailed description of the origin and fate of the follicle sacs of the amphibian ovary. Observations were made on three different amphibians, *Bufo lentiginosus*, *Rana palustris* and *Hyla*. The general description and the drawings (made with a camera lucida) are taken entirely from *Bufo lentiginosus* as I had a much more complete series of this species. As the relatively few stages of *Rana* and *Hyla* at my command confirmed the results obtained on *Bufo* in every respect, a separate description of the follicle sacs in these two amphibians seemed unnecessary and is, therefore, omitted. In preparing the material for study, small portions of the ovary were fixed in corrosive-acetic, and then stained on the slide either with iron-hæmatoxylin, or with a mixture of Lyon's blue and borax carmine, as described in a previous paper (King, 2).

The ovary of *Bufo lentiginosus* consists of several distinct compartments, agreeing in this respect with the ovary of various species of frogs, according to the observations of Rathke, Lereboullet, Brandt and Schultze (5). The walls of these compart-

ments are made up of two membranes which, as a rule, are separated some distance, although occasionally they lie so close together that they appear to be completely united. The outer membrane is single, compact, and of nearly uniform thickness throughout the entire ovary. The inner membrane, on the contrary, appears in some places to be very similar in structure to the outer one; in other parts of the ovary it is found to be composed of two thin layers which always lie close together and are connected at frequent intervals. Cells with very large nuclei are closely applied to the outer and inner surfaces of these membranes and are also present in the spaces between them. (Figs. 3–6, *O. W.*)

The eggs develop between the two membranes composing the wall of the ovary. As they increase in size, they press against the inner membrane of the ovarian wall and cause it to project more and more into the cavity of the ovary. Part of the ovarian

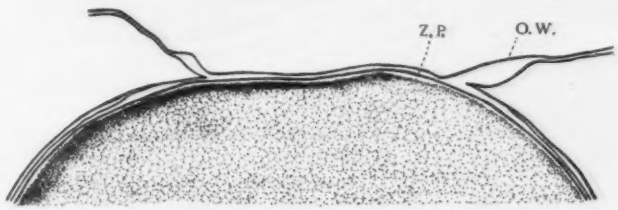

FIG. 1. A portion of an ovarian egg showing the manner of its attachment to the wall of the ovary. Z. P. Zona pellucida; O. W., outer wall of the ovary. Zeiss. obj. A. Oc. 4.

wall comes, therefore, to form a sac that incloses the egg and supplies it with nourishment as long as it remains in the ovary. When the egg is still quite small, there is formed, close to its outer surface, a true egg-membrane, the "zona pellucida" or "chorion," which is thick and seemingly homogeneous in structure (Fig. 2, *Z. P.*). This membrane which is formed, probably, by the numerous follicle cells that are scattered all over the surface of the egg, surrounds the egg during its growth and maturation periods and is invariably found around all eggs taken from the body cavity. Numerous cells and occasionally blood corpuscles (Fig. 2, *C.*) are found between and under the two layers

of membrane which form the outer covering of the egg. These cells, which are usually oblong in shape, contain a small amount of faintly staining, granular protoplasm, and a very large, rounded nucleus with one nucleolus. The chromatin is either collected in masses along the nuclear wall, or it is in the form of a more or less regular network scattered throughout the nucleus.

Ordinarily, when the egg of *Bufo lentiginosus* is ready to leave the ovary, it has a diameter of about 1.5 mm. and from 0.60–0.70 mm. of its surface is in contact with the outer membrane of the ovarian wall (Fig. 1). In the great majority of cases, the egg is attached at the equatorial region as in Fig. 1; but it is not uncommon to find among the eggs thus attached, one with its black or its white pole against the outer membrane.

FIG. 2. Part of an ovarian egg highly magnified, showing the structure of the membranes surrounding the egg. C, blood corpuscle; Y, yolk; Z.P., Zona pellucida. Zeiss apoc. 2 mm. Oc. 8.

By what means the ovarian wall is ruptured and the eggs set free into the body cavity is not known. This process cannot be connected with the breaking down of the germinal vesicle, because the nucleus degenerates and the first polar spindle is formed some time before the egg leaves the ovary, as I have shown in a previous paper (2); neither can it be due to the way in which the eggs are attached to the ovarian wall, for, as I have stated above, the mode of attachment varies with individual eggs. Apparently the eggs must reach a definite stage of development before they break away from the wall of the ovary, because, although I have

sectioned a large number of eggs taken from the body cavity, I have never found a single one in which the polar spindle was not fully formed with the chromosomes arranged along the spindle fibers. Brandt suggests that there may be an active con-

FIG. 3. Longitudinal section of a follicle sac just after the egg has passed into the body cavity. C, blood corpuscles; O. W., ovary wall. Zeiss obj., D. Oc. 4.

traction of the follicle sac when the egg is ready to leave the ovary, and that this contraction stretches the base of the follicle and causes a rupture. How such a contraction takes place without the presence of muscles in the walls of the follicle sac is not at all clear. It seems to me that the rupture of the ovarian wall is more likely to be due to some activity on the part of the egg

itself. Possibly there is a slight increase in the size of the egg at this time which produces sufficient pressure on the surrounding membranes to cause a break at the weakest point. This would seem to be where the egg is attached, because here there are only two membranes over the egg instead of three (Fig. 1). This suggestion, however, fails to account for the rare cases where the egg breaks through its membranes and falls into the cavity of the ovary. Such eggs never leave the ovary; sooner or later they undergo degenerative processes and are gradually absorbed, as are also the few eggs that do not leave the mem-

FIG. 4. Part of the follicle sac shown in Fig. 3 more highly magnified. Zeiss apoc. 2 mm. Oc. 8.

branes at all but remain attached to the ovarian wall. In the latter case the eggs are almost invariably smaller than those taken from the body cavity, and, therefore, retarded development may explain their failure to undergo normal processes.

On spreading out a piece of the collapsed ovary after the eggs have passed into the body cavity, one can plainly see the numerous follicle sacs without the aid of a microscope. These sacs vary in length from 0.36 mm. to 0.54 mm., and their walls are very much wrinkled and folded. Although from 0.60 to 0.70 of

the mature ovarian egg is in contact with the outer membrane of the wall of the ovary, I have never found the opening of an empty follicle sac to be more than 0.38 mm., the average being 0.20 mm. This shows that the edges of the opening tend to close in as soon as the egg has passed out of the ovary.

On examining a longitudinal section of an empty follicle sac (Fig. 3), one finds that the two layers of which its walls are composed are united at frequent intervals, and that, on the wall and in the spaces between the layers, there are numerous cells.

FIG. 5. A portion of the wall of a follicle sac after degenerative changes have begun. Zeiss. apoc. 2 mm. Oc. 8.

Sections of the small blood vessels which supplied the eggs with nourishment are also found occasionally (Fig. 3, *C*). The cavity of the follicle sac is lined with cells, several layers deep in places, particularly at the closed end of the sac. These cells are doubtless the ones that lay between the zona pellucida and the outer covering of the egg at an earlier period, and their confinement in a much smaller space must explain why they appear so numerous, as I have never found any evidence of cell division at this time.

A part of the wall of a follicle sac more highly magnified is shown in Fig. 4. The cells lining the cavity of the sac are seen

to be very closely packed together, and there is rarely an intercellular space to be found. These cells have the same characteristics as those shown in Fig. 2; namely, a very large nucleus containing one nucleolus, a small amount of granular protoplasm, and chromatin partly in the form of a network, and partly in masses scattered along the wall of the nucleus.

On examining the ovary of a toad killed about three weeks after the eggs had been laid, one finds that the openings of the follicle sacs into the body cavity have closed, and that the outer membrane of the ovarian wall is again continuous. Each follicle sac appears at this time as a blind pouch projecting from the inner side of the ovary. The cells lining the cavity of the sac are beginning to show the degenerative changes that are very marked in the ovaries of toads killed the latter part of May. The most noticeable changes are those which are taking place in the nucleus (Fig. 5). Although at this time the nucleus is still round or oval, its outline is somewhat more irregular than it was at an earlier period, and most of its chromatin is collected in from one to four rounded masses that stain black with iron-hæmatoxylin and a deep red with the borax carmine and Lyon's blue mixture used. The protoplasm of the cells appears very granular, and it stains so feebly that it is often very difficult to determine the cell outlines. Such changes, as a rule, are first apparent in the innermost cells that soon break away from the rest and become scattered throughout the cavity of the follicle sac. These free cells never show any pseudopodia; on the contrary, they are more rounded than are the cells still attached to the walls of the follicle sac, and it is therefore impossible to determine whether they have the power of independent movement or not.

Although I have examined the ovaries of many toads killed during the month of June, I have not been able to determine whether the cells lining the follicle sacs are destroyed by the leucocytes, as are the eggs that fail to leave the ovary, or whether they disintegrate and are absorbed by the cells of the ovarian wall. In all the toads killed at this time, either the cells within the follicle sacs were undergoing degenerative changes similar to those shown in Fig. 5, or else the cavity of the follicle sac was nearly obliterated and no traces of the cells could be found. It

is evident that the final stages in the disappearance of these cells take place very quickly and that a large amount of material would be necessary in order to trace their entire history. A longitudinal section of one of the follicle sacs found in the ovary of a toad killed on the fifth of June is represented in Fig. 6. The sac measures 0.13 mm. in length and is, therefore, about one third its original length. The walls of the sac are thinner and

FIG. 6. A longitudinal section of a degenerating follicle sac taken from the ovary of a toad killed on the fifth of June. Zeiss apoc. 2 mm. Oc. 4.

much less folded than they were at an earlier period, and its cavity has nearly disappeared. Numerous cells are found closely applied to the walls of the sac at this time, but they show no degenerative changes, and are undoubtedly concerned in the absorption of the follicle sac itself. Much smaller structures of the same general character as that shown in Fig. 6 can be seen in the ovaries of toads killed the latter part of June; but after this time no traces of them can be found.

The follicle sacs, having served the important function of attaching the eggs to the walls of the ovary and of supplying them with nourishment during their growth period, are of

no further use after the eggs pass into the body cavity. They therefore undergo rapid degeneration and absorption to make room for the young eggs that are in the process of development.

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THE MOVEMENTS OF THE ENTEROPNEUSTA AND THE MECHANISM BY WHICH THEY ARE ACCOMPLISHED.

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The observations upon which the present paper is based were made, so far as they pertain to the living animal, upon two species of the group found on the Coast of California and Washington. These are *Balanoglossus occidentalis* Ritter (MS.) and *Delichoglossus pusillus* Ritter (MS.). The first mentioned is abundant at Puget Sound, and is found, though in small numbers, at San Pedro, California. The second is widely distributed along the Coast of California and is exceedingly abundant at San Pedro, where it lives side by side with the first.

My study of the movements have been made mostly on *D. pusillus*. This small species is so abundant in limited areas at San Pedro that a single shovelful of the sand in which it lives may contain several dozen. Its place of greatest abundance is a large sand flat, exposed at low tide, just inside the mouth of the inner harbor at Timm's Point. The sand in which the animals live is compact and homogeneous and contains much fine argillaceous material, the whole being blackened by decaying organic matter. In some places the creature is found among the roots of scattered patches of eel grass. Although the species is closely related to *D. Kowalevskii* of the Atlantic Coast of the United States, it is considerably smaller. It never produces the coiled castings so characteristic of its eastern relative. In fact, I have failed to discover any marking on the surface of the sand that can be relied upon to reveal the presence of the creature. The absence of the casts which are such a convenience to the collector is, however, compensated by the animal's habit of the protrusion of the proboscis from the ground. At low tide large numbers of the orange-colored proboscises may be seen with the tips only, or sometimes with nearly the entire organ, lying flaccid on the sand. A slight jarring of the ground causes a prompt withdrawal of the exposed part. Professor B. M. Davis, of the Los Angeles State

Normal School, first called my attention to the fact that the protrusion of the proboscis occurs only while the sand is uncovered during low tide ; that withdrawal takes place almost immediately upon the incoming of the tide. My own observations confirm this. I have several times searched the ground in which the animals are abundant, when it was covered by a few inches only of still, clear water, and have never under such conditions seen a single extruded proboscis. The meaning of this habit is not clear.

Movements of both boring and locomotion are effected in the enteropneust through a combination of ciliary and muscular action. When crawling about on the surface of objects, which it does with considerable facility, the ciliary method is chiefly, though by no means exclusively, depended upon. When boring, or moving up and down its canal, muscular action is on the other hand most used.

The best way to observe the movements is to allow the animals to bury themselves in sand contained in a glass vessel. Under such circumstances they are certain to move along the inner surface of the glass if the vessel be small (a test-tube or homeopathic vial, for example, I have found favorable), for a portion of the time, and the whole operation may then be easily watched.

By far the most vigorous activity in any portion of the animal, both ciliary and muscular, goes on in the proboscis. The ciliary action is seen to best advantage when the animal is moving about on a sandy surface on which there are small elevations with which the tip of the proboscis comes in contact now and then ; for the creature does not readily bring its proboscis tip into contact with a perfectly even surface on which it may rest. As the end of the proboscis touches the slope of a sand hillock, a perfect flood of sand grains is at once started which soon wholly encircles the organ and passes rapidly back to the collar. Arrived here the rapidity of the flow is greatly lessened so that if the picking up of grains at the tip be prevented a girdle of sand is soon formed around the collar.

If, on the other hand, the acquisition of grains be not interrupted the whole proboscis and collar become coated by a continuous sheath of sand which gradually extends backward until

the whole animal is covered, the sheath being scarcely more than one or two grains in thickness. The backward movement of the sand on the collar and body is effected chiefly by the pushing force of the ciliary action of the proboscis, though a weak ciliary action on the collar and thorax also contributes to the result, as does likewise muscular contractions of the proboscis. The prolific secretion of mucus particularly by the proboscis and collar serves to agglutinate the sand grains, to produce the sheath above mentioned. This sheath forms a well-defined tube when produced under the conditions here described so firm as to support itself in pieces of considerable size when pulled off and held up in the water.

Simultaneously with the ciliary action the animal is undergoing almost constant contortions through muscular action; but the true nature and significance of the motions of this kind are not seen until the creature is observed in the processes of burrowing. Here the meaning of a characteristic movement of the proboscis which is constantly noticed when the animal is not buried is readily seen. I refer to the contraction waves that move along the organ from tip to base, but which often remain stationary in the form of great blebs.

When in its burrow these blebs act chiefly as hold-fasts by which, through the contraction of the longitudinal muscles of the proboscis and collar, the whole body is drawn forward. The muscles most concerned in this are first those of the proboscis; second, the radio-longitudinal muscles of the collar; and third, the longitudinal muscles of the thorax-abdomen.

The proboscis tip is driven forward in making a new burrow partly by the action of the cilia, and partly by the contraction of the layer of circular muscle fibers, the former apparently playing the more important part.

The wave motion so characteristic of the proboscis does not appear on the collar, thorax, or abdomen, so that it plays no part in the advancement of these portions of the body. The collar shortens and elongates without great change in diameter, though the anterior rim is sometimes narrowed down considerably, the possible change in length of the part being nearly or quite one half its entire length in the fully extended state. The shortening is un-

doubtedly effected almost wholly by the contraction of the radio-longitudinal muscles. The extension is probably accomplished, as Spengel believes, by the inflation of the collar cœlom with water through the collar funnels.

Observation on the movements of the animal furnishes little satisfaction to one's quest after a reason for the existence of the peduncle that joins the proboscis and collar. At the same time, however, such observation does emphasize the importance of the slender isthmus's being both strongly constructed in itself and also firmly anchored at the ends to the larger parts of the body into which they are inserted. As the animal draws its long body about by its proboscis, it frequently looks as though the slender peduncle would be pulled out by the roots, particularly at its collar end. One naturally conjectures that the existence of the peduncle is to increase mobility at this particular place, and this perhaps is its significance, though it is not clear that the peduncle is in reality more flexible than the proboscis, especially when the latter is perfectly flaccid, for I have seen this part when the animal was in its canal double back upon itself so as to bring the two limbs into contact throughout their length, the tip of the organ being carried back to its base. The collar and thoracic portions of the body, however, are always, and the proboscis when muscular contractions are in progress, relatively rigid; and it is probably under these conditions that the mobility assured by the peduncle, finds its importance.

Having now the facts before us relative to the movements of the animal, we may consider the anatomical structures upon which these depend.

The proboscis possesses a typical dermo-muscular tube and its movements are essentially of the wave form usual to this type of musculature. The close resemblance between the movements of the enteropneust proboscis and those of the nemertean may be especially mentioned.

The thorax-abdomen, also, may be regarded as possessing the dermo-muscular tube, though here circular muscles are either altogether wanting or are very imperfectly developed, so that the wave movement does not here take place. Furthermore the longitudinal muscles are much stronger in the ventral than in the

dorsal half of the body in these regions. Ventral curvature as well as fore-and-aft shortening is consequently a general result of the contraction of these muscles.

The chief interest in the myology of the animal centers in the collar and peduncle. Here we have no dermo-muscular tube, but, on the contrary, a musculature of a fundamentally different type. The muscles, both longitudinal and circular, of the dermo-muscular tube are always, it will be recalled, strictly somatic, *i. e.*, they belong to the *body wall*. The chief mass of muscles of the collar and peduncle, viz., the radio-longitudinal muscles, while attached to the body wall at one end, at the other are on the other hand attached to the *notochord* and *nuchal skeleton* mainly, but partly also to the *wall of the esophagus*.

The full details of the origin, insertion, and relations of these large muscles are too complicated to admit of being fully set forth otherwise than by an elaborate, well-illustrated description; but an understanding of the most important facts may be given by a brief presentation, particularly if reference be made to Spengel's monograph. Figures 11, Pl. 14, and 20, 28, 32 and 34, Pl. 15, *lmi.* marking the muscles under consideration, are especially clear and instructive in this connection.

The two muscle masses are bilaterally situated and are in general coextensive in length with the collar and peduncle together. Their origins are on the sides of the combined notochord and nuchal skeleton anteriorly, *i. e.*, in the peduncle; and on the esophageal notochord and esophagus itself posteriorly, *i. e.*, in the collar (compare Figs. 4 and 7, *i. l. c. m.*, Pl. VII., of the author's paper on *Harrimania maculosa*[1]).

From these extensive origins the fibers extend backwards and outwards, some to become inserted into the outer edges of the septum separating the thoracic and collar cœloms; some, with a more oblique course, to insert into the ectoderm or the underlying connective tissue, along the postero-lateral portions of the collar; and still others at the anterior end of the collar, with a course predominantly radial, to insert into the ectoderm of the

[1] Papers from the Harriman Alaska Expedition, II. *Harrimania maculosa*, a new genus and species, etc. *Proc. Wash. Acad. of Sciences*, Vol. II., Aug. 20, 1900, p. 111.

anterior rim of the collar. By the contraction of these muscles when the proboscis acts as a fixed point through the pressure of the blebs above described against the sides of the burrow, the collar and, of course, the whole pharyngeal-abdominal portion of the body, are drawn strongly forward. Following, or coincidently with, this movement, contraction of the longitudinal muscles of the last-mentioned parts themselves takes place which contributes still further to the advancement of the creature as a whole. Circular somatic muscles are practically wholly wanting in the collar. A few fibers have been described in some species, but I am unable to recognize any excepting a few in the anterior rim, in any of the species examined by me.

From the account here given it is seen that we have in these animals *a system of locomotor muscles acting on an axial skeleton, derived in large part from the digestive tract.* That the combined notochord, consisting of the proboscis and esophageal portions, and nuchal skeleton, must be interpreted as a *unit of structure with the office of an axial skeleton,* I hope to be able to show conclusively in my forthcoming monograph of the Enteropneusta of the Pacific Coast. I imagine, however, that the proposition will be readily granted by most zoölogists, particularly if the facts here briefly set forth be considered along with others that may be gathered from previous publications on the anatomy and development of these animals.

If now the considerations relative to the skeleton and muscles be regarded from the comparative standpoint, the most striking thing that comes to view is the trenchancy with which they separate the enteropneusta from all the invertebrata. Nowhere in all the variety of organization represented by this subkingdom do we find locomotion accomplished by *muscles attached either to the intestinal tract or to derivatives of it.* But the trenchancy with which the structural relations here considered separate the enteropneusta from the invertebrata is no greater than that by which they connect them with the chordata.

An axial skeleton derived primarily from the intestinal tract and serving as the attachment of muscles of locomotion is, as is fully recognized, one of the very essences of chordate organization; and in spite of the difficulties in the way of establishing a

complete homology point by point between the axial skeleton (as I have defined it) of the enteropneust with its appended radio-longitudinal muscles of the collar, and the axial skeleton of amphioxus with its attached system of muscle plates, I can but believe that the two systems must have had, far back, a common origin.

But the problem is too large to admit of extended discussion in the present communication. My immediate purpose will have been attained if I shall have called the attention of zoölogists to the undoubted fact, as it seems to me, that the *muscular relations in the collar region of the enteropneusta must receive consideration from a point of view that has not hitherto been held, in all future attempts to elucidate the kinships of this group of creatures.*

UNIVERSITY OF CALIFORNIA,
August 28, 1902.

EMBRYONIC AND REGENERATIVE DEVELOPMENT IN PLANARIANS.

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The term "regenerative development" may be applied to the formation of a new from a part of a preëxisting individual whereby certain structures are carried over essentially unchanged from parent to daughter. In the following paper regenerative and embryonic development in *Planaria maculata* are compared.

EMBRYONIC DEVELOPMENT.

The classical account of the embryonic development of planarians is given in the excellent paper of Iijima, published nearly twenty years ago.[1] Iijima studied more especially the development of *Dendrocœlum lacteum*. The development of *Planaria maculata* seems to be essentially similar. With the early embryonic stages down to the formation of the "embryo-pharynx" we are not at present concerned. It is the transformation of the spherical embryo, filled with yolk cells, into a worm of normal structure and proportions that is of special interest from the standpoint of regenerative development.

At the period when the imbibition of yolk cells begins to be active the planarian embryo is spherical in form. It is surrounded externally by a well-marked membrane formed of greatly distended, flat epithelial cells, shown in section in Fig. 2, *A*. Between this membrane and the yolk cells which fill the central cavity is a reticular substance in which are scattered large cells with nuclei containing one or more nucleoli. These cells are represented by the larger bodies in the body-wall shown in Fig. 2, *A*. One of them is shown in Fig. 4, *a*. The nucleus is immediately surrounded by a granular mass of protoplasm. The reticular substance of the body wall probably represents the

[1] Iijima: "Unters. uber die Bau u. die Entwicklungsgesch. d. Süsswasser-Dendrocœlen (Tricladen)," *Zeitschrift f. Wissenschaftliche Zoölogie*, Vol. XL., 359, 1884.

syncytial "ectoplasm" of these cells. Cell division at this stage is mainly by mitosis. In addition to the cells just described many small nuclei, granular masses, and vacuoles are embedded in the substance of the body wall. These bodies I take to be remnants of yolk cells. Most of the small nuclei show marked evidences of disorganization. Some of the large cells of the body wall lie flattened against the yolk cavity but I can find no evidence at this stage of a differentiation between entoderm and mesoderm cells like that described by Iijima for *Dendrocœlum*. The embryo-pharynx corresponds closely in structure to that described by Iijima.

As the embryo becomes distended by taking in yolk cells the body wall becomes thinner and the character of the cells lying in the reticular substance is altered. The nucleus becomes more densely granular and division takes place mainly by amitosis (see Fig. 4, *b*). The protoplasm of these cells varies in granulation and in staining power, but I have been able to distinguish no clearly defined classes of cells. Rapid cell division gives rise to a smaller variety of cell shown in Fig. 4, *c* and *d*. These cells exhibit indications of amœboid movement, as shown in Fig. 4, *d*. In these smaller cells mitotic division prevails, though amitosis is also present.

The hitherto spherical embryo now becomes flattened dorso-ventrally and elongated in the antero-posterior axis. This change in form is accompanied by a more rapid cell-multiplication in the ventral than in the dorsal body wall. The most rapid cell-multiplication takes place on the postero-ventral wall just below the embryo-pharynx (see Fig. 2, *B*). It is from this mass of cells that the permanent pharynx is developed. Iijima says that in *D. lacteum* the permanent pharynx is developed in the region of the embryo-pharynx after the disappearance of the latter. The relations between embryonic and permanent pharynxes in *Planaria maculata* were first discovered by Winterton Curtis and are described by him in an article shortly to appear in the *Proceedings* of the Boston Society of Natural History.

At the period of the formation of the anlage of the permanent pharynx tissue-differentiation becomes active. Before considering this tissue-differentiation it may be best, however, first to go over

in brief outline the assumption by the embryo of the form characteristic of the adult.

In Fig. 1 are shown four stages in the development of the embryo. These figures were sketched by means of a camera lucida from living specimens. The outline of the intestinal cavity, as it appeared by transmitted light, is shown by serrated lines. In *D* the septa extending into the intestinal cavity are represented more highly developed than is common at this stage.

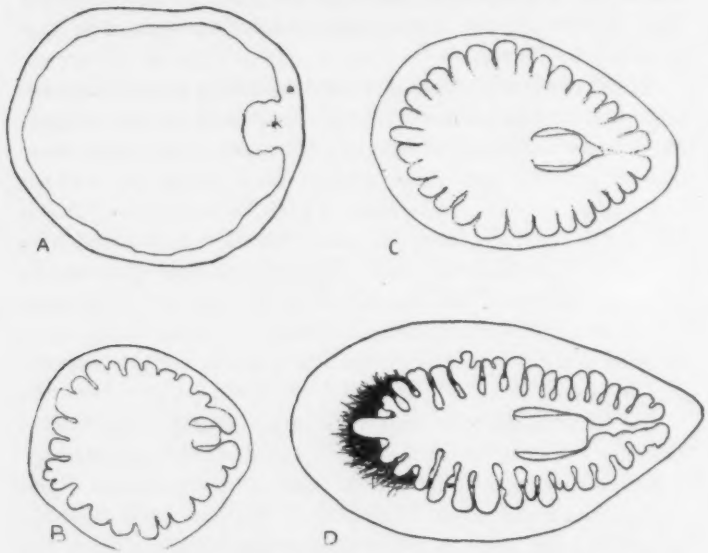

FIG. 1. Various stages in the development of the embryo. Magnification, 57 diameters.

A. Dorsal view of an embryo in which the anlage of the permanent pharynx is being developed. At the right the opening into the embryo-pharynx is shown.

B. Ventral view of an embryo in which the pharynx has begun to assume definite form.

C. Ventral view of an older embryo in which the two posterior extensions of the intestinal cavity have united behind the pharyngeal pocket.

D. Dorsal view of an embryo at the period when the central nervous system has assumed definite outlines.

This was done so as to expose the nervous system. The latter was reconstructed from sections made from the embryo. In *A* the opening into the embryo-pharynx is shown at the right. In

B the permanent pharynx is shown near the posterior end of the piece. This individual seems to have been a small one at the time of metamorphosis. In *C* and *D* the development of the tail posterior to the pharyngeal region is shown. There is a temporary anastomosis of the posterior rami behind the pharyngeal pocket. This anastomosis later disappears, as shown in Fig. 1, *D*. The head is meanwhile developed, as shown in the figures, anterior to the primitive yolk cavity.

The relations of the head, pharyneal and tail regions to the primitive yolk cavity of the embryo are also represented in Fig. 2. In *B* the anlage of the permanent pharynx is shown. The

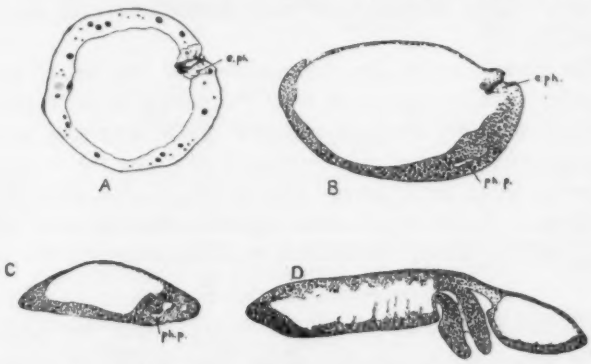

FIG. 2. Sagittal sections in the median line of embryos of various stages of development. Magnification, 57 diameters.

A. Section through an embryo which is becoming filled with yolk cells.

B. Section through an embryo in which both the embryo-pharynx and the anlage of the permanent pharynx are seen.

C. Section through an embryo in which the two posterior extensions of the intestinal cavity are uniting behind the pharyngeal pocket.

D. Section through an embryo in which the tail region is well developed and the anlage of the head is well marked. Owing to the longitudinal contraction caused by the killing fluid the pharynx has been partly forced out from the pharyngeal pocket. *e.ph.*, Embryo pharynx. *ph.p.*, Pharyngeal pocket.

base of the pharynx extends obliquely from the region of the embryo-pharynx to the ventral wall. In *C* the base of the pharynx still extends obliquely from the dorsal to the ventral walls. The embryo-pharynx has disappeared. A tail region posterior to the pharynx and a head region anterior to the yolk cavity are

distinctly developed. In *D* the assumption of adult condition is much more advanced. The base of the pharynx now extends nearly perpendicular from the dorsal to the ventral body wall. Head and tail regions are well developed.

The first indications of a definite nervous system appear in the region of the head at about the stage of development shown in Fig. 1, *C*. The outlines of the nervous system are, however, at this period too indefinite for satisfactory illustration. There are apparently bilateral anlages soon united by a commissure. The lateral cords gradually extend from the region of the brain towards the tail. At the period shown in Fig. 1, *D*, the central nervous system has the form there shown somewhat diagrammatically.

The median nerves and commissures and the lateral nerves probably arise by outgrowth from the central nervous system. Nothing is known of the development of the peripheral plexus.

Reproductive organs are developed only after the worm has advanced far in development. Curtis, (*op. cit.*) has followed the development of the reproductive organs in *Planaria maculata*. I shall therefore attempt no description of the process in this place.

Histogenesis in the Embryo.

The development of the various tissues during the formation of the structures characteristic of the adult is difficult to follow. For the sake of making a comparative study of the size and structure of the various cells at different stages of development I have used a uniform method of technique. The organisms were first anæsthetized with chloretone [1] to cause relaxation and to prevent the pre-mortem contraction which killing fluids are apt to stimulate. They were then fixed in a one-half saturated solution of corrosive sublimate to which acetic acid was added, carried through alcohol solutions of increasing strengths up to absolute alcohol, and thence into xylol. The tissues were embedded in paraffine, cut into sections $3\frac{1}{3}$ to $6\frac{2}{3}\,\mu$ thick, and stained in dilute Delafield's hæmatoxylin, followed by a solution of Congo red in water. A little Congo red was also added to the alcohol used in dehydration. The figures have been drawn to a fixed scale by means of the camera lucida.

[1] A term applied commercially to chloroform-acetone.

The surface epithelium is the simplest of the tissues to follow, owing to the sharp line of demarcation existing between it and the underlying tissues. Up to the time of the formation of the anlage of the permanent pharynx the surface membrane is beset but sparsely with cells. Fig. 3, *a*, shows one of these cells in cross-section. During the period of assumption of adult structure by the embryo these cells multiply rapidly by amitosis, as shown in Fig. 3, *b*. The cells increase in thickness, Fig. 3, *d*.

FIG. 3. Sections illustrating various stages in the development of the surface epithelium. Magnification, 540 diameters.

a. From the embryo shown in Fig. 1, *A*.

b. From an embryo in which the pharyngeal anlage is being formed.

c. Dorsal epithelium near the pharyngeal region of an embryo in which the nervous system is beginning to appear.

d. Epithelium from the ventral surface of the tail region of the same embryo.

e. Dorsal epithelial cells from an adult individual.

f. Ventral epithelial cell from an adult individual.

g. Epithelium covering a cut surface of an adult individual twelve hours after injury.

In *b* and *c* evidences of direct nuclear division may be seen.

Finally the membrane becomes covered externally with cells so crowded together that the long axis of each cell lies at right angles to the surface of the body, Fig. 3, *c*. This crowding first takes place near the center of the dorsal and ventral surfaces, where bodily extension is least rapid, and only later at the growing extremities of the worm (compare *c* and *d*, Fig. 3). The dorsal epithelium at no time is ciliated (Fig. 3, *c*), the ventral epithelium acquires cilia at an early stage of development (Fig. 3, *d*). Although it is commonly stated in the text-books that fresh-water planarians are completely covered with cilia when

young, I have seen no specimens of *Planaria maculata* at any stage of development in which the dorsal epithelium was ciliated. For the sake of comparison a dorsal and a ventral epithelial cell from an adult individual are shown in *e* and *f* of Fig. 3. These seem to be slightly smaller than the embryo cells. Although the possibility cannot be excluded that cells are added to the surface epithelium from the parenchyma, I have found no evidence of this.

The cells of the parenchyma offer a most difficult and uncertain subject for study. Cells of the type shown in Fig. 4, *e*, are

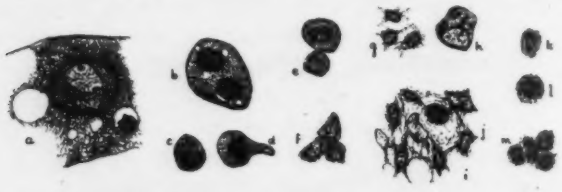

FIG. 4. Sections illustrating the development of the parenchyma. Magnification, 540 diameters.

a. One of the cells surrounding the yolk cavity of the embryo shown in Fig. 1, *A*.

b. Corresponding cell of a slightly older embryo.

c. Type of cell prevailing in the parenchyma of an embryo immediately before the stage when the pharyngeal anlage appears.

d. Similar cell showing evidence of amœboid movement.

e. Cells illustrating the reduction in size which takes place during the tissue differentiation that occurs at the time when the pharyngeal anlage appears.

f. Closely packed cells seen in the anlage of the permanent pharynx.

g. Connective-tissue cells of body wall during this stage.

h. Early rhabdite cell.

i. Connective-tissue cells of adult individual.

j. Large parenchymal cell in resting stage.

k, *l*. Large parenchymal cells undergoing division.

m. Cells from anlage of head thirty-eight hours after isolation of the piece.

those most commonly met with at the stage preceding the development of the permanent pharynx. In certain regions, such as the pharyngeal anlage, these cells may be very closely packed together (Fig. 4, *f*), but if we assume them to form a syncytium we must consider it a syncytium in which the cell territories are fairly well marked. The individual cells may be irregular in shape or distinctly elongated. From cells of this character

smaller cells with smaller nuclei arise and form a syncytium of branched cells (Fig. 4, *g*). The connective tissue of the adult is derived from cells of this nature (Fig. 4, *i*), fibrils in the processes connecting the cells becoming ever more distinct. In addition to these fixed connective-tissue cells there exist in the parenchyma of the adult several other types of cells. Some of these, with dark protoplasm, large nuclei, and well-marked nucleoli lying in clear areas, seem to be the direct, undifferentiated descendants of the embryonic cells of the type shown in Fig. 4, *e*. These cells divide by mitosis (Fig. 4, *k* and *l*) and seem to be the chief factor in the regeneration of new parenchyma. Other cells with lighter nuclei and protoplasm the granules of which stain deeply in "acid" dyes may be leucocytes, as Miss Stevens has aptly suggested.[1] Cells of this nature are seen at an early stage and apparently are derived from cells of the type shown in Fig. 4, *e*. Rhabdite cells begin to be differentiated even before the period when the epithelium assumes a columnar structure, but while this latter process is taking place the rhabdite cells approach the basement-membrane and rhabdites are passed out into the intercellular areas. In Fig. 3, *c*, the depression in the membrane indicates the area through which rhabdites have been passed. I have seen no clear indications of the passage of the rhabdite cells themselves through the membrane. Mucus cells and the gland cells belonging to the intestinal system arise at a comparatively late stage. I have not been able to trace their origin from any specific cells.

When lining surfaces like the pharyngeal pocket the parenchyma cells assume an epithelial character. These cells have an origin similar to that of the connective-tissue cells.

Intestinal cells become clearly evident at the time when the embryo assumes an ovoid form. They are at first sparingly scattered, but at the time when septa extend into the yolk-cavity so as to give rise to the permanent form of the intestinal system, they begin to multiply by indirect division (Fig. 5, *a*). As in the case of the surface epithelium, this cell multiplication finally gives rise to cells extending away from the base on which they rest

[1] "Notes on Regeneration in *Planaria lugubris*," *Archiv f. Entwickelungsmechanik der Organismen*, XIII., p. 410, 1901.

(Fig. 5, *b*). As pointed out by Iijima, however, this last process is slow, and it is only at a comparatively late p_riod in development that the intestinal epithelium assumes the structure characteristic of the adult. Whether the intestinal epithelium is differentiated at a very early period and then preserves its specificity, like the surface epithelium, or is added to by constant additions from the parenchyma, it is difficult to decide. The former view seems to me perhaps more probable. It must be stated in

FIG. 5. Sections illustrating the development of the intestinal epithelium. Magnification, 540 diameters.

a. Flat cells seen in embryos at the time of the development of the permanent pharynx.

b. Cells beginning to project toward the lumen of the intestine in an embryo in which the nervous system has just become clearly visible.

c, *d*. Intestinal cells undergoing direct division during regeneration in the adult.

e. Cells bordering on the intestines. In one cell a mitotic figure is visible.

this connection, however, that the parenchyma cells bordering on the intestines show active proliferation by mitosis during embryonic development as well as during regeneration (see Fig. 5, *c*).

Myoblasts become numerous at about the period of the formation of the anlage of the permanent pharynx (Fig. 6, *a*). They are very large cells and apparently are derived from cells of the type shown in Fig. 4, *c*, many of which have more vesicular nuclei than there shown. The myoblasts seem to divide by indirect division. From those lying next the basement membrane of the epithelium is derived a syncytium, a portion of which is shown in Fig. 6, *b*. In this the surface musculature is developed. It is probable that a separate syncytial sheet exists for each layer of musculature. Other myoblasts give rise to the muscle fibers which run through the parenchyma in various directions.

Blochmann,[1] who studied the tissues of cestodes by means of the methylen-blue and silver impregnation methods, describes as "Sommer-Landois cells" large cells lying below the surface musculature and sending out branches connected on the one hand with the fibers of the surface musculature and on the other hand extending into the peripheral nerve plexus. The muscle fibers he

FIG. 6. Sections illustrating the development of the musculature. Magnification, 540 diameters.

a. Myoblasts in an embryo in which the pharyngeal anlage is being formed.
b. Developing circular musculature at a later stage.
c. Developing parenchymal muscle cell.
d, *e*. Small nuclei lying between the muscle fibers of an adult individual.
f. Large cell occupying a corresponding position.
g. Developing circular musculature during regeneration.

considers outgrowths of processes of these cells. In *Planaria maculata* large cells with vesicular nuclei, resembling in size and form the myoblasts of the embryo, may be seen lying below the surface musculature. Whether or not these represent Sommer-Landois cells which have migrated from the syncytial musculature after the formation of the latter cannot be decided by methods which I have employed. Monti has described cells of the Sommer-Landois type in planarians.[2]

It is uncertain whether the musculature of the pharynx arises from the cells potentially differentiated at the time of the formation of the pharyngeal anlage.

Of all the tissues the nervous tissues are the most difficult to follow in these animals. Only by special technical methods can this be satisfactorily done and yet the planarian tissues seem very refractory to the ordinary impregnation methods. Blochmann (*op. cit.*) used these successfully on cestodes and gives an inter-

[1] "Ueber freie Nervenendigungen und Sinneszellen der Bandwürmern," *Biologisches Centralblatt*, XV., 14, 1895.

[2] *Archives Italiennes de Biologie*, Vol. XXVII., p. 15, 1897.

esting description of the elements of the peripheral nerve plexus. In this many nerve cells are found. Monti[1] has stained by the Golgi method and described unipolar, bipolar and multipolar cells lying both in and about the brain and nerve cords and in the periphery next the musculature but she has not followed their development. Bergendal[2] found in the central nervous system of land planarians large ganglion cells, with very large nuclei which stain lightly.

I have made no extended attempt to stain the nervous system of planarians by special methods. In and about the nerve-cords and brain are scattered nuclei that stain very lightly and are much larger than the nuclei of the fixed connective-tissue cells (see Fig. 7, *e*, and compare with Fig. 4, *i*). These I have taken to be the nuclei of nerve cells. The cell-body is usually difficult to follow, but at times this may be seen sending off one or more

FIG. 7. Sections illustrating the development of the nervous system. Magnification, 540 diameters.

a. Large cell seen in the anterior ventral region of an embryo before the anlage of the pharynx has appeared.

b. Similar cells after the anlage has appeared.

c. Early stage in the development of nerve fibers.

d. Nerve cells in an adult individual.

e. Row of nerve cells seen in a regenerating nerve cord.

f. Nuclei of nerve cells undergoing direct division during regeneration of a new brain.

processes. At the stage of development in the embryo immediately preceding the formation of the permanent pharynx a few large, palely staining cells may be seen in the anterior portion of the ventral body wall. A cell of this kind is shown in Fig. 7, *a*. I have not found these cells elsewhere in these embryos and think it possible that they represent neuroblasts. I have tried to find them in earlier stages of development without success. They

[1] "Sur le system nerveux des Dendroceles d'eau douce," *Archiv. Ital. de Biologie*, Vol. XXVII., 15, 1897.

[2] *Zoölogischer Anzeiger*, X., 218–224, 1887.

do not seem at this period to arise from the surface epithelium. At the time of the formation of the anlage of the permanent pharynx, cells like these in a corresponding region may be seen multiplying by direct division (Fig. 7, *b*). When the brain anlages first become clear, owing to the formation of nerve fibers, similar cells may be seen lying among the fibers (Fig. 7, *c*). In the nerve cords, as they grow toward the tail region, corresponding nuclei may be seen, often in columns and often appearing to divide by direct division. It seems possible, therefore, that the nerve cells of the central nerve system of these planarians may arise by direct division from neuroblasts which are differentiated in the anterior ventral wall of the embryo at a comparatively early period, and that the other cells of the central nervous system may arise by direct division from these.

The cells of the peripheral nerve plexus may arise, however, in the periphery and not from those of the central nervous system. In the adult many very pale nuclei may be seen scattered here and there below the musculature. These nuclei resemble those of the cells found within the central nervous system.

During embryonic development, therefore, we find that the surface epithelial cells are derived by direct division from the preexisting ectoderm cells. The intestinal cells certainly, and also probably the nerve cells, multiply by indirect division. Whether additions are made from parenchyma cells after the primary period of differentiation is uncertain. The early myoblasts multiply partly, at least, by indirect division. The period at which the differentiation of myoblasts from the parenchyma ceases is undetermined. Branched, anastomosing connective-tissue cells, "leucocytes," rhabdite cells and gland cells are differentiated in the parenchyma but certain cells retain a size and form characteristic of an early stage of embryonic development.

Regeneration in Embryos.

When removed from the egg-capsule embryos of the stages represented in Fig. 1, *A* and *B*, and younger ones live but a short time in water. Twenty-four hours is the longest I have been able to keep them alive. Embryos of the stage shown in Fig.

1, *C*, may, with care, be kept alive until adult form is assumed; but if mechanically injured they do not long survive and pieces removed from them will not live. If embryos of the stage shown in Fig. 1, *D*, be cut in a region immediately anterior to the pharynx both anterior and posterior pieces may sometimes be kept alive. The anterior piece will develop a new pharynx, but the posterior piece will not develop a new head, although it may otherwise undergo full development, including the assumption of the pigmentation characteristic of the adult. It seems probable, although it cannot be taken as proved, that the failure of the posterior piece to give rise to a new head is due to the absence in the piece of well-formed nerve cords. If the cut be made near the head or in older embryos a new head may be regenerated.

In *Dendrocœlum lacteum*[1] F. R. Lillie found that a new tail may be formed by outgrowth from any transverse level behind the region immediately back of the eyes, but a head only on a cut surface made in the anterior third of the body. These results have been confirmed by E. Schultze[2] and by me. Lillie ascribes the result to the cephalization of the nervous system in *D. lacteum*. It is interesting to note that at the period when the central nervous system is "cephalized" in *Planaria maculata* similar conditions prevail.

Regenerative Development.

For the sake of comparing embryonic with regenerative development we may consider briefly the regeneration of new individuals from cross pieces taken from in front of the pharynx, from tail pieces and from slips removed from a region lateral to the median line.

Cross Pieces.—There is a close similarity between the development of a new individual from a cross piece cut from in front of the pharynx and development in the embryo. In the cross piece the pharynx develops on the posterior ventral surface, with relations to the axial gut similar to that borne by the pharynx to the yolk cavity in the embryo (see Fig. 8). The head and tail re-

[1] "Notes on Regeneration and Regulation in Planarians," *American Journal of Physiology*, Vol. VI., p. 128, 1901.

[2] "Aus dem Gebiete der Regeneration bei Turbellarien," *Zeitschrift für wissenschaftliche Zoölogie*, Vol. LXXII., p. 1, 1902.

gions are likewise developed in a manner resembling the embryonic.

If the cut surface on which the new head is developed be at right angles to the long axis of the parent worm the new head will be developed symmetrically about the anterior extremity of the axial gut, just as it is developed symmetrically about the anterior extremity of the yolk-cavity in the embryo. If, however, the cut be made oblique, the head may arise some distance anterior and lateral to the region where the old axial gut projects on the cut surface. Morgan first described this phenomenon.

FIG. 8. To illustrate the development of a new individual from a cross piece 48 hours after removal. Magnification, 57 diameters.

A. Individual as seen from the dorsal surface. The intestinal system was sketched from the living individual, the nervous system was reconstructed from sections.

B. Sagittal section in the median line from the same individual. *ph.p.*, Pharyngeal pocket.

Fig. 9 shows a good example from *Dendrocœlum lacteum*. The median ends of the lateral intestinal branches lying in the piece anterior to the extremity of the old axial gut and separated from the latter by the cut have become so anastomosed as to form an extension of the old axial gut. About the anterior extremity of this the new head is being symmetrically differentiated. In the further growth of the piece new tissue is rapidly formed to the right of the axial gut and about its extremity until the worm is restored to normal proportions.

Tail Pieces. — In tail pieces regeneration of the new head takes place symmetrically about a branch extending forward from the area of fusion of the two posterior rami of the original piece. In all of the instances which I have examined this fusion has taken place before differentiation of a head has really begun. The process of head formation resembles essentially that described as taking place on the anterior surface of the cross piece taken from in front of the pharyngeal region. The formation of the pharynx

is the most puzzling process in the regeneration of these tail pieces.

The region of pharynx formation depends upon the condition of the piece and the region of the cut. In individuals not sexually mature if the cut be made posterior to the old pharynx the new pharynx will develop slightly anterior to the center of the piece (Fig. 11, *B*). I have followed the development of a large number of such specimens during a cool spell when the regenerative processes were somewhat slow, and the successive stages in regeneration therefore well marked. The following notes in-

FIG. 9. To illustrate the formation of a new axial gut lateral to the axis of the original gut, after oblique section; *D. lacteum*. Magnification, 17 diameters. The regenerated intestinal branches are shown in black.

FIG. 10. To illustrate the development of a head symmetrically about the end of the axial gut and posterior to an anterior lateral slip which contained no nervous system. Magnification, 17 diameters.

dicate these stages. The number of hours given in each instance shows the length of time elapsing after isolation of the pieces:

Eighteen hours. The cut surface is covered with epithelium and there is some accumulation of parenchyma cells near it.

Thirty-eight hours. Further accumulation of cells near the cut surface.

Sixty-six hours. Anastomosis of the posterior intestinal rami in the vicinity of the cut surface (Fig. 11, *A*). Advance of nerve fibers into the head anlage. In some specimens there is a slight accumulation of cells in the vicinity of the future pharynx.

Ninety hours. The accumulation of cells in the pharyngeal anlage is more marked. Between the region of anastomosis of the posterior rami and the pharyngeal anlage new intestinal branches are being formed. The head anlage has advanced in development.

One hundred and fourteen hours. Considerable anastomosis

between the intestinal branches last mentioned. In some specimens a new axial intestine had thus been formed between the anlage of the head and that of the pharynx (Fig. 11, *B*). The pharynx is beginning to be differentiated. The head is much advanced in development but the chief commissure of the brain is not completely formed.

One hundred and thirty-eight hours. The axial intestine and pharynx are well formed. The chief commissure of the brain is well formed and the eyes have appeared.

Subsequently the axial intestine formed between the two original posterior rami becomes greatly developed at the expense of the latter.

Sometimes the process is more simple than that described above, the anterior portion of one or the other of the posterior rami becoming converted directly into an axial gut; or fusion of two rami may be more complete, as in the instance described in my previous paper.[1] Miss Stevens (*op. cit.*) states that the accumulation of cells for the pharyngeal anlage may begin in *P. lugubris* before intestinal anastomosis takes place. We must admit, therefore, that in instances of this kind the pharyngeal anlage is not formed in response to stimuli arising from a definite axial intestinal cavity, as seems to be the case in the embryo and in pieces cut anterior to the pharynx, but rather upon the relations borne by a given area of the worm to the intestinal system as a whole.

In sexually mature worms cut immediately anterior to the genital apparatus the pharynx is formed much further anterior than in sexually immature specimens. The reproductive organs undergo retrograde metamorphosis. Destruction of the genitals begins at the periphery of these organs where the intestines border upon them and extends towards the center (see Fig. 11, *C*). The process is a complex one which I shall not attempt to describe in this place. The ducts and glands, including the testicles, also become disorganized, the last to disappear being those near the posterior end of the animal. As a result of the breaking down of these organs the neighboring tissues of the planarian

[1] "On the Physiology of Regeneration in *Planaria maculata* with Especial Reference to the Phenomena of Regeneration," *American Journal of Physiology*, Vol. V., p. 1, 1901.

become filled with bits of disorganized tissue. Most of this eventually finds its way into the intestines which become thereby so distended that in many places the lining epithelium is as thin as that found during embryonic development. Stevens (*op. cit.*) has described a similar process in *P. lugubris*. The pharyngeal anlage is formed in the median line immediately anterior to the degenerating sexual apparatus and posterior to the center of the anastomosis between the posterior rami (Fig. 11, *c*).

If the cut isolating the tail piece pass through the pharyngeal pocket the new pharynx will develop at the anterior end of the

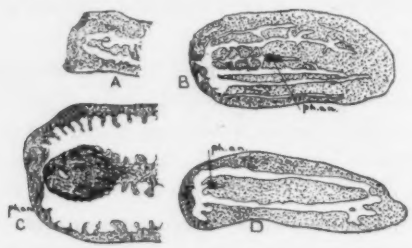

FIG. 11. To illustrate the development of new individuals from tail pieces. Magnification, 17 diameters.

A. Intestinal anastomosis, sixty-six hours after isolation of the piece.

B. Intestinal anastomosis ninety hours after isolation of the piece. The pharyngeal anlage is indicated, *ph.an*.

A and *B* are from individuals not sexually mature.

C. Piece from an individual sexually mature. The reproductive organs are undergoing degeneration.

D. Tail piece isolated by a section passing through the pharyngeal pocket.

pocket. If a portion of the tip of the old pharynx be retained in the chamber this tip will be utilized to form the end of the new pharynx.[1]

Lateral Slips.—In lateral slips isolated by a cut lateral to the region of a lateral nerve cord no specific regeneration takes place. This, at least, is the result I have obtained not only in *P. maculata* but also in *P. lugubris*, *Dendrocœlum lacteum* and *Phagocata gracilis*. If a part of the lateral cord be present in such a

[1] The process of retrograde metamorphosis of the retained portion of the pharynx which I previously described (*op. cit.*, p. 34) is the exception, not the rule. If a portion either of the base or the tip of the pharynx be retained in the piece it will usually be utilized in the regeneration of the new pharynx.

slip a new head will be regenerated, and if a fairly considerable amount of the intestinal apparatus be contained in the slip, a new pharynx will be formed. The intestinal apparatus, if it originally consists of isolated parts, tends to become anastomosed into a whole. A new head is formed about that portion of the intestinal system which projects on the cut surface in the neighborhood of the most developed portion of the nerve-cord (see Fig. 12, *A*). The new pharynx is formed posterior to the region of least bodily compression, polarity being understood to be determined by the nervous system. If the lateral slip be cut so that

FIG. 12. To illustrate the development of an individual from a lateral slip. Magnification, 17 diameters.

A. Piece isolated from the region lateral to the pharyngeal pocket.

B. Two individuals developing from a lateral slip from the center of which a portion of the nerve cord was removed.

two or more regions will be formed in which the nervous and intestinal apparatus of each section will be isolated from that of the neighboring section, double or even triple individuals may be formed. Fig 12, *B*, indicates a double individual of this kind.

Having thus briefly considered the regeneration of new individuals from these typical isolated pieces, we may now take up, first, the question of histogenesis during regeneration and, second, that of the conditions determining the formation of definite structures.

HISTOGENESIS DURING REGENERATION.

After removal of the piece the surface epithelium spreads out so as to cover the cut area with a layer of epithelium like that covering the embryo, but somewhat thicker than the latter (see Fig. 3, *g*). Stevens (*op. cit.*) gives an excellent account of this process in *Planaria lugubris*. Direct cell division restores the epithelium to normal form. The cells regenerated on the dorsal surface are not ciliated.

The most active cells in the development of the parenchyma of the new part are cells which closely resemble the cells playing a similar part in embryonic development (Fig. 4, *e*, *k*, *l*) and, like them, they divide by indirect division. These cells probably represent the "Stammzellen" mentioned by Keller.[1] The many branched connective-tissue cells seem also to assume a spherical form and to multiply by indirect division. The new connective tissue (Fig. 4, *m*) resembles the embryonic (Fig. 4, *f*). The new surface musculature may arise from the large cells shown in Fig. 6, *f*, cells which resemble the embryo myoblasts. The musculature of the parenchyma seems, however, to arise from cells of the type shown in Fig. 4, *h* and *l*, as do also the rhabdite cells.

The epithelium lining the intestinal branches which extend into the new parts apparently multiplies by direct division (Fig. 5, *c* and *d*).

Cells bordering immediately upon the intestines multiply by indirect division (Fig. 5, *e*). I am uncertain whether these cells contribute to the supply of the intestinal epithelium, but am inclined to think that they do not. Stevens (*op. cit.*) thinks that they may. The new nervous system arises by direct extension of fibers and apparently also of cells from the old into the new parts. Fig. 7, *e*, represents a growing bundle of cells and fibers of this nature. Fig. 7, *f*, shows direct nuclear division in the vesicular nuclei of the cells which accompany the fibers into the new parts. At present, however, no certain evidence can be offered that parenchyma cells do not also become directly transformed into nerve cells under the influence of the old nerve cords. The majority of investigators believe that the nervous system is differentiated from the parenchyma, though in direct continuity with the old nervous system.[2]

The reproductive organs seem to arise from the cells of the type shown in Fig. 4, *e*. During their development mitosis is

[1] "Die ungeschlechtliche Fortpflanzung der Süsswasser Turbellarien, *Jenaische Zeitschrift f. Naturwissenschaften*, Vol. XXI, p. 370, 1894.

[2] Flexner, *Journal of Morphology*, Vol. XIV., p. 337, 1898. Stevens, *op. cit.* Morgan, "Growth and Regeneration in *Planaria lugubris*, *Archiv für Entwickelungsmechanik*," Vol. XIII., p. 179, 1901. E. Schultz, "Aus dem Gebiete der Regeneration bei Turbellarien," *Zeitschrift für wissenschaftliche Zoölogie*, Vol. LXXII., p. 1, 1902.

active. E. Schultz (*op. cit.*) has given an excellent account of the regeneration of the reproductive organs in *D. lacteum*. Morgan has shown (*op. cit.*) that in *P. lugubris* pieces cut anterior to the old reproductive organs may regenerate them.

An exceptionally good discussion of histogenesis in forms allied to the planarians may be found in the article by Keller (*op. cit.*).

Factors Determining Development.

Having thus considered the origin of the tissues entering into the new parts, we may now consider the factors concerned in the development of the various regions in planarians. Of these regions the most definite and fundamental are the head and pharynx. In the embryo the head is developed at one extremity of the yolk-cavity, the pharynx at the other. Between these two regions the yolk-cavity is converted by the ingrowth of septa into an axial gut with lateral branches. The axial gut grows forward into the developing head and posteriorly it sends two branches into the caudal region which develops behind the pharynx.

In regeneration we likewise find that pharynx and head are developed with certain relations to the intestinal system of the isolated piece. Brought near to one another by muscular contraction isolated intestinal branches become anastomosed into a unit system. By making certain cuts it is possible to have two or more such unit systems developed in a single piece, as shown in Fig. 12, *B*. Head and pharynx are developed in connection with such a unit system only when there is contained within the piece a portion of the central nervous system. This is shown in Fig. 10, where the unit intestinal system of the anterior lateral slip has neither head nor pharynx developed in connection with it. Furthermore, the piece containing the unit system must be above a minimum size. Thus in very short head pieces and short, narrow lateral slips no pharynx may be developed although the piece contain a portion of the original intestinal system and a portion of the original nervous system.

What determines the location of the anlage of the pharynx? In a previous paper (*op. cit.*) I showed that the pharynx arises in a region posterior to that portion of the intestinal system into

which, on general bodily contraction, the contents of the whole intestinal system tend to be forced. I called this portion of the intestinal system the area of "least intestinal pressure" but this term is bad because it admits of several interpretations. The area into which the intestinal contents tend, on general contraction, to be forced, and in which the new pharynx arises, is essentially the area of least bodily compression. In *P. maculata* the stimulus to pharyngeal formation is exerted in an antero-posterior direction in this region. Polarity in the piece is determined by the central nervous system. The pharyngeal anlage first appears near the ventral wall of the body.

In the embryo it seems fair to assume that the contents of the yolk-cavity are forced toward the embryo-pharynx on general contraction. Closure of this would cause a stimulus to pharynx formation to be exerted on the body wall comparable to that exerted in regenerative development.

In cross pieces taken from in front of the pharynx and in lateral slips the relation of pharynx to intestines is fairly clear (Fig. 8 and Fig. 11).

In tail pieces the problem is more complicated. When no reproductive organs are present the pressure exerted within the anterior extremities of the posterior intestinal rami by contraction of the piece tends to force the intestinal contents into the anterior median branches of the posterior rami, and thus to give rise to an intestinal area not at first occupied by a single intestinal cavity, or axial gut, but by several distended branches. Posterior to this area pharyngeal formation may be started even before the median branches of the posterior rami have fused to form an axial gut. When, however, a portion of the pharyngeal pocket or the sexual organs are contained in the piece the anterior median branches of the posterior rami are thereby prevented from expanding so that the intestinal contents are forced further forward into the area anterior to the pharyngeal pocket or to the reproductive organs.

The exact nature of the stimulus giving rise to pharyngeal formation is still in the dark, although we can point out the area where it seems to be exerted. It is fairly certain, however, that bodily fluids are forced in considerable quantity into the area of the pharyngeal anlage.

In *Phagocata gracilis* F. R. Lillie[1] has shown that in the pharyngeal anlage a considerable number of pharyngeal pouches are developed and that these subsequently fuse. In lateral slips the pharyngeal pouches on the cut side are formed only subsequently to the development of an intestinal branch on that side. In this animal the pharyngeal area seems to be determined by factors similar in nature to those at play in *Planaria maculata*, but pharyngeal formation in this area is limited not to a single stimulus exerted posteriorly from the region of the axial gut, but, on the contrary, may be excited by stimuli arising from anterior median branches of the chief posterior rami of the developing intestinal system. For a criticism of the view which I have expressed as to the factors determining the seat of pharyngeal formation the reader is referred to Lillie's article.

The pharynx may be formed either near a cut surface or quite within the body of the piece. A new head is formed only in the tissue produced on a cut surface. This surface may be a flat plane. In such a case the median axis of the head will at first be perpendicular to the cut surface. Or the head may be produced at the junction of two planes. Thus, if the anterior end of the worm be removed by a V-shaped cut, the angle of the V pointing posteriorly, and the two sides be allowed to unite, as a rule a new head will be produced, the median axis of which is in line with the chief axis of the worm. If they be not allowed to unite a head will be produced on each side with its median axis perpendicular to the plane of the cut. If the V point anteriorly sometimes a head will be produced on each cut surface; at other times a single head will arise, the median axis of which corresponds with the longitudinal axis of the worm. When a new head arises thus at the juncture of two plane surfaces the median axis of the head seems to bisect the angle formed by the junction of the two cut surfaces. Of course, the cut surfaces may not be planes, but may be very irregular in shape. In all such instances, however, similar axial relations prevail.

The direct stimulus to head and brain formation seems to be due to the presence near the cut surface of the chief coördi-

[1] "Notes on Regeneration and Regulation in Planarians, *American Journal of Physiology*, Vol. VI., p. 134, 1901."

nating area of the nervous system of the piece. In most cases of regeneration of a new head, this means the most anterior portion of the old central nervous system. Lillie has stated this point of view very clearly in the article referred to above. But while the stimulus to brain formation arises from the chief coördinating center of the piece, the head and the brain are symmetrically formed about a center of nutrition which is represented by that portion of the intestinal system which lies immediately adjacent to the nerve center, and which is in most direct continuity with the main intestinal apparatus. A symmetrical head is formed both when the stimulus to brain formation comes from one side and when it comes from both sides of this projecting intestinal tip.

There is an interesting correlation between the lateral nerve cords and the eyes. In oblique pieces the most direct stimulus to brain formation comes from that nerve cord which is the more anteriorly situated. As a rule the eye on the side corresponding to this nerve cord is developed earlier, and at first larger, than that upon the other. This is still more marked in the regeneration of oblique cross pieces divided in the median line, as shown by Morgan (*op. cit.* 1901). So, too, in the regeneration of lateral slips the eye on the outer side is usually earlier developed than that on the median.

That stimulus to brain formation depends upon the central nervous system projecting into the area of the cut and that the head is formed symmetrically about the tip of the axial gut, with the cut surface as a base, is indicated by the following experiment. If a longitudinal cut be made lateral to the nerve cord from the region of the head back nearly to the pharynx, and then transversely across the body, the slip thus left attached to the main piece will contain none of the central nervous system of the animal. The slip will contract posteriorly and will bend over so as to become fused to the transverse cut surface. When regeneration is well under way it will be found that the new head is developing on the transverse cut surface, symmetrically about the tip of the old axial gut, as shown in Fig. 10. The contracted anterior slip exhibits no specific influence on the regeneration of the head, although the latter is continuous with

it. Subsequently, normal form is usually restored by forward growth of the head region and absorption of the lateral slip. By making two such anterior lateral slips, one on each side, it is possible to get a head to develop posterior to a ring of tissue formed by the anastomosis of these slips. As the head grows it projects dorsally in a forward direction beyond the anastomosing lateral slips.

The influence exerted by the intestinal apparatus upon the regenerating part is probably in the main a nutritive one. Owing to the action of the musculature upon the intestinal system that region of the periphery of the body which receives the richest nutritive supply is probably the area about the tip of the axial gut where the head is differentiated. Owing to its proximity to the axial gut the whole anterior regenerating region also receives a rich nutritive supply and this may account for its rapid growth until normal proportions are reached.

The effect of proximity to the axial gut on regenerating tissue is illustrated by the following experiment.

If a narrow longitudinal slip of tissue be removed from one side of the anterior half of the body and a broad slip from the other, regeneration of tissues will be the more rapid on that side from which the broad slip has been removed. This area of more rapid regeneration lies nearer the axial gut.

Morgan (*op. cit.*, p. 200) gives a picture of an oblique cross piece in which the anlage of a new head has appeared before intestinal anastomosis is visible between the pharyngeal area and the base of the head. Morgan traced the outlines of the intestinal system of the piece from the pigment particles contained within the intestinal cells. I have previously shown, however (*op. cit.*, p. 22), that but little pigment is found in the newly formed anastomotic intestinal branches, so that in the piece mentioned by Morgan an anastomotic axial gut may have existed. By using small, slightly pigmented, starved worms in which the reproductive organs are not developed the intestinal outlines may be fairly accurately followed during life, if the pressure of a coverglass be used to flatten the specimens. Oblique illumination is often of aid. In the hundreds of generating pieces of planarian which I have examined, I have never seen an instance in which

differentiation of a head has taken place otherwise than about the tip of an intestinal branch connected with the main intestinal apparatus of the animal. This intestinal branch may be termed the axial gut because of its subsequent relations to the regenerated worm. Lillie, in a valuable contribution to the subject of regeneration in planarians (*op. cit.*, p. 141), states that "the intestinal system regenerates in relation to the new external parts and not *vice versa*, as maintained by Bardeen; from which it follows that the location of the new parts cannot be due primarily to the form of the gut." As a matter of fact it is probable that mutual relations exist between the intestinal system of the animal and the regenerating parts, each exerting specific influences upon the growth of the other.

After the head and pharynx have been formed, the processes which serve to restore the worm to normal proportions become very marked and, unless certain conditions prevail, such as lack of size or attachment to another individuality (as shown in Fig. 12, *B*), this is soon effected. Bilateral symmetry, however, may arise in partially detached pieces containing neither head nor pharynx. Morgan has made a special and valuable study of the general gross relations borne by an isolated part to the regenerated individual.[1]

In small pieces isolated from sexually mature worms the reproductive organs at first completely disappear, as shown above in discussing regeneration in tail pieces. They are regenerated only after the worm has reached a certain advanced stage of development. In *Planaria maculata*, furthermore, the development of the sexual organs seems to depend upon season. In *Dendrocœlum lacteum* Iijima found that after depositing the cocoons the individuals die, but this is not true of specimens of *P. maculata* kept in captivity. It is probable that there is great variation in different species of planarians in the time of development and the stability of the reproductive organs. Schultze, as mentioned above, gives an excellent account of the regeneration of the reproductive organs in *D. lacteum* (*op. cit.*).

[1] *Op. cit.*, and also "The Internal Influences that Determine the Relative Size of Double Structures in *Planaria lugubris*," BIOLOGICAL BULLETIN, Vol. III., p. 132, 1902.

The destruction of the genital apparatus in isolated tail pieces shows that in the reorganization which takes place during regenerative development highly differentiated tissues, like the musculature of the genitals, are not transformed into new parts, but instead undergo retrograde metamorphosis and serve probably as food stuff for the other tissues. On the other hand, highly organized parts may be utilized directly in the formation of new organs. This is shown by the union of a tip of the pharynx retained in the pharyngeal pocket to a regenerated base.

Summary.

1. The anlage of the pharynx is formed at one extremity of the yolk-cavity of the embryo, that of the head at the other.

2. Up to the period of the formation of the pharynx the yolk-cavity is surrounded by a layer of parenchymal cells and these by a thin epithelial sheet. The parenchymal cells multiply by direct and by indirect division and become reduced in size as they increase in number.

3. The ectodermal cells begin to multiply rapidly by direct division after the pharynx has been formed, and thus give rise to a columnar epithelium. The dorsal cells at no time develop cilia, while the ventral cells produce them before becoming columnar in form.

4. Tissue differentiation begins in the parenchyma shortly before the pharyngeal anlage appears. From the parenchyma are developed intestinal cells, muscle cells, nerve cells, rhabdite cells and gland cells, as well as branched connective-tissue cells and possibly leucocytes. Certain of the parenchyma cells seem to multiply without undergoing further differentiation. Intestinal, nerve and muscle cells multiply by direct division, while the parenchyma cells multiply mainly by indirect division.

5. The central nervous system is first developed in the region of the head and from there the lateral cords grow posteriorly.

6. When removed from the cocoon very young embryos soon die. Slightly older ones die if injured. After the pharynx has become functional the embryos may be cut into two or more parts which will live. Pieces from which the head has been removed will not develop a new head unless they contain well-developed nerve cords.

7. In regeneration the new ectodermal cells arise from the old by direct division. New nerve cells, intestinal cells and muscle cells seem likewise to arise from preëxisting cells by direct division. It is possible, however, that they may spring from the large cells of embryonic type situated in the parenchyma. From the latter the other tissues mainly arise.

8. The new pharynx arises immediately posterior and ventral to the region into which the intestinal contents are forced by general muscular contraction and in response to stimuli arising from this region.

9. A new head is regenerated only in tissue produced at a cut surface, and with definite axial relations to the surface. The direct stimulus to head formation arises from an exposed chief coördinating region of the central nervous system. The head is formed with radial symmetry about the tip of the main intestinal branch extending to the cut surface in the vicinity of the exposed nervous system.

10. After head and pharynx have been differentiated the activities tending to restore the piece to a worm of normal form and proportions become especially marked.

11. During regeneration highly differentiated tissues are destroyed unless they may be directly utilized in the formation of new parts.

A CASE OF ABNORMAL PLUMAGE.

R. M. STRONG.

The purpose of this paper is to describe a case of abnormal plumage. The material was obtained from a hybrid pigeon belonging to Professor C. O. Whitman, which was reared at Woods Holl, Mass., in the summer of 1902. The male parent of the bird was a ring dove (*Turtur risorius*) and the female parent was a hybrid between a male little red ring dove of China (*T. humilis*) and a common ring dove (*T. risorius*).

The abnormalities under consideration occurred in the plumage succeeding the *natal* down, the so-called *juvenal* plumage of Dwight (:00), and they were of three distinct types, belonging respectively to the remiges, the rectrices and the body coverts.

FIG. 1. Dorsal view of secondary from left wing of hybrid pigeon having abnormal plumage. × 1.

1. *The Remiges.* — The remiges all had a transverse band of slightly paler color across the proximal portion of the distal half of the vane (see Fig. 1). The shaft, barbs and barbules were all more sparsely pigmented where this band occurred, and some of the barbules were represented only by stubs. This condition of the barbules is particularly noticeable at the proximal ends of the barbs whose insertions on the shaft occur in this region, and it will be considered in the description of abnormalities in the rectrices. The vane was also slightly narrower at this point. The position and character of this band were uniform in all of the remiges, though there were very slight variations in its paleness.

2. *The Rectrices.* — The rectrices were also crossed by a transverse band in a position similar to that noticed in the remiges, but the region is much more abnormal. The loss in pigmentation is greater than was the case in the remiges, and

there is an area of almost complete absence of normally formed barbules (Fig. 2). The barbules of both the distal and the proximal rows in this region were represented only by stubs of the proximal portions, and the stubs in the distal row were much shorter than those of the proximal row (Fig. 3). To the unaided

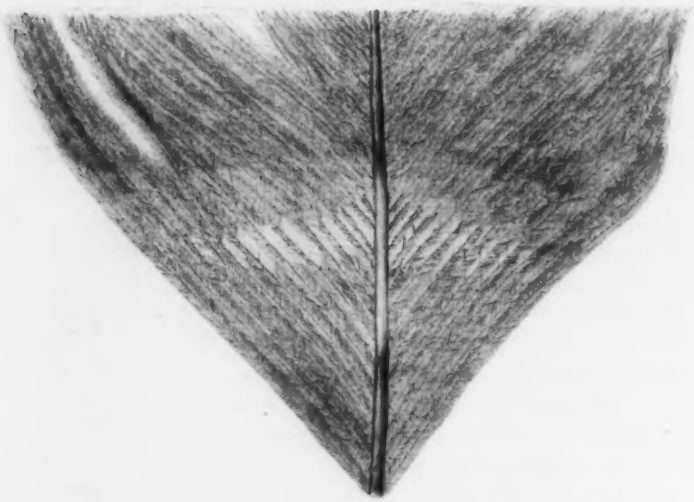

FIG. 2. Dorsal view of the abnormal region in one of the rectrices, the fourth from the left side of the tail. × 6.

eye the vane appears loose in texture at this point and, as in the remiges, it is slightly narrower here.

It has been impossible to determine the histogenesis of these barbule stubs, but it seems to the writer unlikely that they represent the entire structure produced in the feather germ. The occurrence of barbules of normal appearance among the stubs with no intermediate conditions and the irregular fractured appearance of the distal ends of the stubs point strongly towards the conclusion that a distal portion has been broken away. There are a number of species of birds which normally have feathers with the barbules broken off at certain fairly definite points near their proximal ends in the distal portions of the more distal barbs as has been observed by Meves ('55), Chapman ('96), Dwight (:00),

and others, including the writer. The barb itself may be broken near the distal end according to Meves and Chapman. In these instances, the breaking is believed to occur some weeks or months after the feather was fully developed. In the case of these abnormal feathers, however, nothing is known as to the time when the barbules may have been broken, but the condition was observed only a few days after the feathers had emerged from the sheath enclosing the feather germ.

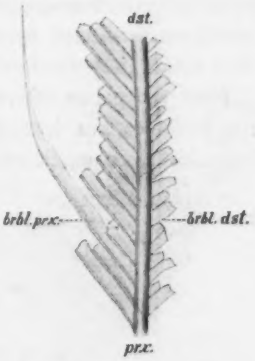

FIG. 3. Dorsal view of a portion of a barb bearing barbule stubs from the left side of the shaft in the region represented in Figure 2. × 74. *brbl. dst.*, distal barbules; *brbl. prx.*, proximal barbules; *dst.*, distal end of portion of barb figured; *prx.*, proximal end of portion of barb figured.

The Body Coverts.—The most striking type of abnormality appeared in the body coverts, and it is very different from what was seen in the remiges and rectrices.

A normal body covert of the juvenal plumage from another hybrid is shown in Fig. 4. The downy portion, *i. e.*, the part where the barbules are long and with rudimentary barbicels is represented diagrammatically. The barbs or portions of barbs bearing shorter barbules with hooked barbicels usually, in the distal row, are indicated semi-diagrammatically by lines radiating from the shaft, but no attempt has been made to represent the true number of the barbules. It will be seen that by far the larger portion of the vane of such a feather is downy.

An abnormal feather from a corresponding region on the back of the bird under consideration is represented in Fig. 5. Almost the whole feather appears downy and there is a strange tassel-like appendage to the distal end of what may be called the main body of the feather. With the aid of a microscope, one finds that two or more of the more distal barbs are fused at their distal ends into a horny, unpigmented, irregularly-shaped cylinder (Fig. 5, *cyl.*), which branches at its distal end into several downy barbs and a median shaft that itself bears barbs on either side in

the usual manner. The shaft of the main portion of the feather is not continuous, however, with the shaft of the distal appendage. The distal ends of the barbs nearest those actually fused with the horn cylinder have strips of horn tissue (Fig. 5, *a*.), which seem to have been torn loose from the cylindrical mass with which they were probably fused in the feather germ.

Near the points where the barbs fuse with this horn cylinder, the barbules are less highly differentiated. The most distal barbules are comparatively irregular strips of keratin which may

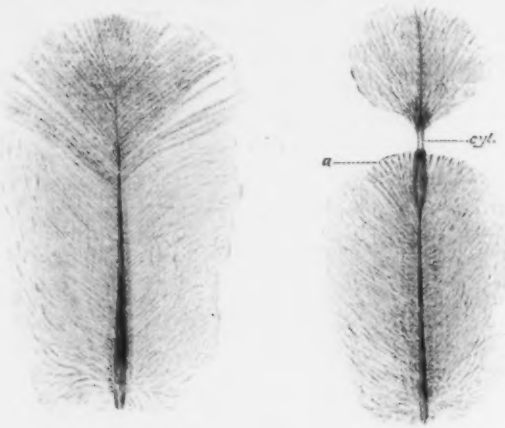

FIG. 4. FIG. 5.

FIG. 4. Dorsal view of a normal body covert from the back immediately anterior to the upper tail coverts. × 2.

FIG. 5. Dorsal view of an abnormal body covert from a region corresponding to that furnishing the feather shown in Figure 4. *cyl.*, horn cylinder; *a*, position of a fragment torn from horn cylinder. × 2.

be fused at their distal ends with the cylindrical mass, as can be seen in Fig. 6 where two barbs and a barbule are shown fusing with a strip of tissue torn from the cylinder.

Discussion and Conclusions. — It is significant that these abnormalities occur at uniform distances from the distal ends of the feather throughout the whole plumage, and it seems reasonable to conclude that the conditions responsible for the abnormalities were constitutional, and affected the germs of all the feathers

simultaneously, though in three different degrees of intensity. The length of the period during which these conditions obtained is, presumably, approximately that required for the differentiation of a portion of the shaft equal to the band shown in Fig. 1.

Professor Whitman tells me that he once had a young common domestic dove which developed similarly abnormal feathers. This bird was underfed during the period when the *juvenal* plumage was developing. I am inclined to accept Professor Whitman's suggestion that in both cases the abnormalities are due to malnutrition. The effects upon the remiges and rectrices were comparatively slight, and appear simply as a weakening in pigmentation and structure, but the body coverts were strikingly modified.

FIG. 6. Dorsa view showing fusion of two barbs and a barbule into a strip from the cylinder of undifferentiated cornified tissue which connects the distal appendage shown in Fig. 5 with the main body of the feather. × 49.

The horn cylinder (Fig. 5, *cyl.*) is just such a structure as one would expect to find if the processes of differentiation in the fundament of the barbs and barbules, which have been described by Davies ('89), Strong (:02), and others were to be arrested temporarily. The shaft, barbs and barbules are developed in apartments of a cylindrical mass of epithelium surrounding a central dermal papilla, and differentiation takes place from the proximal end distally. Epithelial cells proliferated at the proximal end of the feather germ push the differentiating epidermal cylinder distally, and cornification takes place some distance from the proximal end. If the differentiation, but not the cornification, should be omitted, a simple cylinder of cornified tissue such as we see in the abnormal body coverts would result. The appearances at either end of the horn cylinder undoubtedly represent the end of differentiation distally, and at the proximal end (Fig. 6) the resumption of differentiation of the normal feather elements.

I wish here to express my thanks to Professor Whitman for the material described in this paper and for courtesies received in connection with the investigation.

THE MARINE BIOLOGICAL LABORATORY,
WOODS HOLL, MASS., September 4, 1902.

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MATURATION, NATURAL DEATH AND THE PROLONGATION OF THE LIFE OF UNFERTILIZED STARFISH EGGS (ASTERIAS FORBESII) AND THEIR SIGNIFICANCE FOR THE THEORY OF FERTILIZATION.

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I. Introduction.

I have pointed out in my earlier publications that fertilization of the egg serves to prolong the life of the egg.[1]

The mature unfertilized egg dies in a comparatively short time. Because of this fact the egg becomes of importance as an object of experiment, to study the question of natural death and prolongation of life. For by no means has it been decided that there is a "natural" death. We only know that with an increase in age a critical period is reached in which every living organism dies under the influence of conditions which do not affect a younger organism. It may, therefore, be of interest that we are able to show, as I believe, that a critical period exists in the life of many eggs in which they die a "natural" death, and that the life of the eggs can, during this period, be saved or lengthened only through various external conditions.

The egg of the starfish (*Asterias Forbesii*) serves as a very favorable object of experiment in the study of this question. When removed from the ovary this egg is generally "immature," but as soon as it comes in contact with sea water it begins to "maturate."

Morphologically, the immature state is characterized by a very large, plainly visible nucleus.[2] The process of maturation consists

[1] Loeb, J., *American Journal of Physiology*, Vol. IV., 1901; Loeb and Lewis, *ibid.*, Vol. VI., 1902.

[2] The recent beautiful experiments of Delage have shown that, beside these visible changes in the nucleus, chemical, but morphologically invisible, changes also occur in the protoplasm. Delage, Études expérimentales sur la maturation cytoplasmique et sur la parthénogenèse artificielle chez les Échinodermes, *Arch. de Zoologie expériment.*, Vol. IX., 1901.

morphologically in this, that the nucleus becomes invisible and the polar bodies are thrown out.

This process is completed within one or two hours after the eggs are removed from the ovaries and placed in sea water. Only when maturation is complete is it possible to cause the egg to develop through the addition of sperm or through the physical and chemical agencies that have been described by me, Delage, Mathews, and Greeley.

II. The Natural Death of the Mature Unfertilized Starfish Eggs.

The living eggs of *Asterias* are light yellow in color and homogeneous. They retain this appearance during the process of maturation as long as they are alive. They retain this appearance also when they are made to develop through the entrance of a spermatazoön or through the proper chemical or physical means.

If, however, the mature eggs are not fertilized or do not develop, they die in the course of four to twelve hours, and this process of dying is accompanied by a characteristic change in the color of the egg. The egg becomes at first opaque, then almost black, the homogeneous structure of the protoplasm becomes granular. If such a culture of unfertilized eggs is examined under the microscope after twenty-four hours, two kinds of eggs are found, first, the just described dark, dead eggs *which are mature*, and secondly, *living* normally colored *but immature* eggs. For usually not all the eggs that are removed from the ovaries of a starfish maturate at once; many maturate very late, others not at all. It is readily seen *that the immature eggs remain alive for several days until they finally become the prey of bacteria; while the mature eggs become opaque and die in four to twelve hours after maturation has been completed.*

Is the death of the mature but undeveloped egg brought about through internal conditions or through the bacteria contained in the sea water?

A trustworthy way of determining this consists in making sterile culture of the eggs in sea water. This is a relatively simple procedure in the case of starfish. Eight flasks were sterilized, filled with sterilized sea water, and again heated for twenty

minutes on three successive days to 100°C. A female starfish was thoroughly washed externally, an arm was opened, and one of the ovaries removed with sterilized forceps and placed in sterilized sea water. From the thick stream of eggs which at once flowed out of the ovary, a few drops were quickly introduced with a sterilized pipette into each of the sterilized flasks. A second series of eight flasks contained normal sea water, and a few drops of the same eggs were introduced into these flasks also. A third series of flasks were filled with sea water, to each of which were added 2 c.c. of a putrid, foul-smelling culture of old starfish eggs in order to bring about a rapid development of bacteria from the beginning. Each of these flasks also contained eggs from the same culture as those in the sterilized flasks.

That perfect sterilization had been attained in the first eight flasks was proved by the fact that all the flasks remained absolutely clear and cloudless during the course of the experiment, and that three of the flasks which had not been opened are even to-day (after six weeks) absolutely clear, and every egg can be individually recognized. The flasks containing the unsterilized sea water became cloudy already after twenty-four hours, and after two days the eggs had become the prey of bacteria and no individual egg could be recognized. The sterilized flasks which were opened were at all times free from foul odor, while the unsterilized flasks gave off a penetrating stench, often after one, invariably after two days. The microscopic examination of the sea water for bacteria was always negative in the sterilized flasks, always positive in the other flasks. In those flasks to which 2 c.c. of the putrid culture of starfish eggs had been added, bacteria and infusoria were exceedingly numerous from the beginning.

Six hours after the beginning of the experiment one flask of each of the three series was opened and the eggs examined microscopically. The picture was the same in all three flasks. Nearly all the eggs were mature, and a small number of them were opaque or black. But what is of the greatest importance to us is the fact that the percentage of opaque dead eggs was just as great in the sterile culture (if not greater) than in the unsterilized or the infected sea water.

Twelve hours later, that is to say eighteen hours after the

beginning of the experiment, one of the flasks of each of the three cultures was again opened. At this time nearly all the eggs of the sterile culture were opaque or black, and a few were already granular. In the two other cultures an equal percentage of the eggs were opaque. The eggs, therefore, die just as rapidly in the sterilized flasks which are absolutely free from bacteria as in the flasks containing bacteria. Death follows from internal causes, and so rapidly that the few bacteria in the sea water are scarcely able to accelerate the death of the eggs. The eggs have already died from internal causes before the bacteria can attack them in sufficient numbers to threaten their existence.

The flasks which were opened later served only to corroborate what has been said. The experiment was repeated with the same result. Each of the flasks that were opened during the first few days also contained a small number of living transparent eggs. *The latter were, without exception, immature. The experiment, therefore, shows that the mature eggs of starfish die in the course of a few hours, and that the cause of this death cannot be sought in the bacteria of the sea water; and further, that under exactly the same conditions the immature eggs remain alive.*

III. The Chemical Conditions Necessary for Maturation in Starfish Eggs.

Since the eggs of *Asterias* are usually immature in the ovary, but, in part, at least, maturate in the course of one or two hours when introduced into sea water, the suspicion was aroused that some of the substances contained in the sea water brought about the maturation. In order to determine which substance this might be, a series of solutions were prepared having approximately the osmotic pressure of the sea water. The result was so simple that it is not necessary to describe all the experiments here. For it was found that when the eggs are introduced into solutions which contain free hydroxyl ions, maturation soon follows, but that this does not occur in solutions containing no hydroxyl ions. So, for example, the eggs retain their nucleus in a $\frac{5}{8}n$ NaCl solution, or in NaCl solutions to which some potassium or calcium has been added. If, however, .5 to 2 c.c. $n/10$ NaOH is added to each 100

c.c. of such solutions, maturation soon follows; that is to say, the nucleus becomes invisible. Since sea water contains free hydroxyl ions the conclusion is justified that these are one of the causes for the maturation of the starfish egg. It was possible to prove this assumption through further experiments. If a small amount of acid is added to sea water, the free OH ions disappear, and the water becomes acid in reaction (through the addition of 1.5 c.c. or more $n/10$ HCl to 100 c.c. sea water). Immature eggs were introduced directly into sea water to which 1, 2, 3 and 4 c.c. of an $n/10$ HNO_3 solution had been added to each 100 c.c. of sea water. While, as is usual, a large percentage of eggs soon maturated in the normal sea water, maturation did not occur at all in the vast majority or in all the eggs contained in the sea water to which 2 or more c.c. acid had been added. The addition of even 1 c.c. of acid diminishes the number of eggs that maturate. But it is not even necessary to keep the eggs permanently in neutral or acid sea water in order to inhibit maturation. If 4 or 5 c.c. of a $n/10$ HNO_3 solution are added to 100 c.c. sea water, and immature eggs are introduced into such a solution for only about fifteen minutes, relatively few eggs maturate when they are returned to normal sea water. Such acidified sea water does not kill the starfish eggs.

We shall see later that the procedure described here which, when used upon immature eggs, prevents maturation, brings about artificial parthenogenesis when used on mature eggs.[1]

I have, moreover, been able to convince myself of the fact that the eggs which are introduced into acidified sea water in an immature state, can be fertilized by sperm if they finally maturate. It is possibly in harmony with what has just been said that the addition of $NaHCO_3$, or larger amounts of sodium citrate to the sea water accelerates the process of maturation. Free hydroxyl ions are present in the solutions of both substances, and it is possible that their addition to the sea water increases the concentration of the free hydroxyl ions in the sea water.

But the hydroxyl ions are certainly not the only substances in the sea water which favor or cause the maturation of the starfish egg. I soon found that when different specimens of eggs are

[1] Loeb, Fischer, and Neilson, *Pflüger's Archiv*, 1901, Bd. 87.

taken from the same culture, and the percentage of mature eggs is determined, this percentage is subject to the greatest variations. The cause of these variations was soon discovered. For it was found that where the eggs lie together in a heap maturation occurs slowly, but where they lie in a thin layer, maturation occurs quickly. This fact suggested the importance of oxygen for maturation. Where the eggs lie in a heap the appropriation of the oxygen by the superficial layers of eggs prevents the diffusion of the oxygen to those lying deeper.

Experiments were now made in which the oxygen of a small flask containing a small amount of sea water was replaced by hydrogen. When, in such experiments, all the oxygen was entirely removed maturation did not occur in any, or at least the majority of the eggs, in spite of the presence of the hydroxyl ions in the sea water. There are, therefore, at least two substances in sea water which cause or accelerate maturation, oxygen and hydroxyl ions. Possibly other constituents of the sea water are also concerned in the process, but NaCl, Ca, and K have apparently no beneficial effect upon maturation.[1]

It seems, therefore, that the absence of oxygen and hydroxyl ions in the ovaries belongs to the conditions which inhibit maturation of the eggs in the ovary.

IV. The Prolongation of the Life of the Unfertilized Starfish Egg by the Prevention of Maturation.

We have shown above that the mature eggs of a culture of unfertilized starfish eggs die within a short time (which decreases with an increase in temperature), while the immature eggs remain alive a relatively long time. It was necessary now to show that when the maturation of a culture of unfertilized egg of *Asterias* is prevented artificially, the eggs live longer. We begin with the experiment which is technically most simple. The eggs streaming from the ovary are divided into two portions. One portion of eggs is carefully distributed without mechanical agitation, by carefully tipping the vessel, in a thin layer over the bottom

[1] Professor Whitman informs me that the maturation of the eggs of *Clepsine* does not begin until after they are laid. Possibly the oxygen contained in the water is in this case also a necessary condition for maturation.

of the vessel. The vessel must be low and the layer of sea water covering the eggs not too deep, so that the diffusion of oxygen to the eggs can occur with ease. A second portion is introduced with just as great care into a small-calibered glass tube sealed at one end. This glass tube is half filled with eggs, so that one is certain that the lower layers of the eggs in the pipette receive little or no oxygen. It is self-evident that the eggs must be introduced into the tube immediately after being laid. When, after twenty-four hours, the eggs which are distributed over the bottom of the glass dish and which receive a large amount of oxygen are compared with those at the bottom of the glass tube, a striking difference is found between them. The eggs richly supplied with oxygen contain a much larger percentage of mature dead and black eggs than those kept in the lack of oxygen. In the latter the living immature eggs are in the majority, and a part of these maturate when spread out in a thin layer over the bottom of a vessel. These experiments are also well adapted to show that the rapid death of the mature unfertilized sea-urchin eggs is determined through internal conditions and not by the bacteria contained in the sea water. I will cite an example.

One portion of a lot of eggs was spread out in a thin layer over the bottom of a dish; another was heaped in a mass in the same dish. The sea water was the same in both cases. The first portion of eggs maturated in a few hours and were, in less than twelve hours, opaque and dead, while the water was still absolutely clear and without odor of putrefaction. After twenty-four hours the water became putrid and contained many bacteria. Even after three days, when the water was exceedingly foul and cloudy, a portion of the eggs which had lain in a heap, that is to say, without oxygen, *were immature and living*. They were introduced into fresh water and spread out into a thin layer. They maturated and developed into swimming larvæ upon the addition of sperm. It is self evident of course that even immature eggs finally become the prey of bacteria, and so go to pieces in the sea water.

The same experiment can be made in a somewhat more complicated way with pure oxygen and hydrogen. The freshly laid eggs

of a starfish were distributed into two series of eight flasks. The one series of flasks was connected with a hydrogen generator; the other with a tank containing pure oxygen. Before the beginning of the experiment all the air in one of the series of flasks was driven out by the current of hydrogen. During the course of the experiment a vigorous current of hydrogen was maintained. Both series of flasks contained freshly laid immature eggs of *Asterias*. The experiment lasted three days, and from time to time a flask was removed and its contents examined. The eggs which had been exposed to the current of oxygen maturated just as rapidly and as numerously as those in ordinary sea water, and the mature eggs soon died. In the current of hydrogen maturation did not occur in the majority of the eggs, and these remained alive. In the hydrogen cultures a rapid development of bacteria occurred while in the oxygen cultures this occurred to a small degree.[1]

Treatment with acids which, as we have shown above, prevents the maturation of the eggs (without killing them) also prevents their death and disintegration.

Eggs which, without having been in contact with pure sea water, are introduced for ten or fifteen minutes into 100 c.c. sea water plus 4 c.c. $n/10$ HCl maturate very slowly or not at all when they are returned to normal sea water. They also retain, as long as they are immature, the transparent, normal appearance of living eggs until they finally become the prey of bacteria.

It seems to follow from these experiments that the same processes which underlie the maturation of starfish eggs also lead to their death (if they are not inhibited through circumstances which we designate by the term fertilization). I tried to see, now, whether it was also possible to maintain the life of the *mature* egg through lack of oxygen. I indeed obtained in a few cases positive results in this direction. The eggs of a starfish were spread in a thin layer over the bottom of a dish. After three hours seventy-five per cent. of the eggs were maturated. A portion of the mature eggs were carefully introduced into the

[1] Care must be taken in these experiments that the air is thoroughly removed from the sea water in the hydrogen flasks before the eggs are introduced into them. Of course the hydrogen apparatus must also be free from air.

glass tube described above, in which the deeper layers suffered from lack of oxygen. A second portion was introduced into a small flask through which a steady stream of pure oxygen was passed. On the following morning, that is to say, fifteen hours after the eggs were brought into the atmosphere of pure oxygen, the various portions of the eggs were examined. The eggs introduced into the current of oxygen showed in one vessel *98 per cent. mature and dark, dead eggs and 2 per cent. immature living* eggs. The eggs which had remained in normal sea water contained, as before, about 75 per cent. mature eggs, all of which, however, were black and dead, with the exception of a few eggs which had begun to divide,[1] and were living.

The immature eggs were also still living. Upon the other hand, the eggs which had been left in the glass tube in absolute or relatively high lack of oxygen, were nearly all living! This observation seems to show that the same processes which lead to the maturation of the egg bring about its death if they are not inhibited at the right time. In this way the process of fertilization becomes a life-saving or life-prolonging act.

V. Do these Facts Hold for Other Forms?

The question of the relation between maturation and natural death can be studied most beautifully in the starfish egg because it is possible to obtain it in an immature condition, and because maturation follows very rapidly. With sea-urchin eggs conditions are much less favorable, since the egg maturates within the ovary, and since it is difficult to obtain immature eggs during the spawning season. I have, therefore, been unable to discover which chemical factors determine the maturation of the sea-urchin egg, and to decide whether the same circumstances cause the death of the sea-urchin egg that bring about the death of the starfish egg; and whether the life of the sea-urchin egg can be prolonged through a prevention of these circumstances. In an indirect way Lewis and I attempted to answer this question last year, when we assumed that the destructive processes which bring about the

[1] This cleavage was possibly brought about through mechanical agitation; I had repeatedly shaken the dish to facilitate the introduction of oxygen into the sea water.

death of the unfertilized egg are enzymatic (autolytic?) processes which can be inhibited through poisons such as KCN.[1]

We did in fact succeed in showing that the addition of a small amount of KCN to the unfertilized starfish eggs markedly lengthens their life. Even after seven days such eggs can be fertilized as soon as they are returned to normal sea water. We also pointed out that, because of the well-known bactericidal properties of potassium cyanide, the experiments on sea-urchin eggs were not in themselves decisive and so began experiments on starfish eggs[2] which, however, we were not able to complete at that time. In dealing with eggs which are as long lived as sea-urchin eggs a great development of bacteria in normal sea water can not be prevented, since a few of the eggs always die and so serve as an excellent culture medium for the further development of bacteria. It need, therefore, surprise no one that the unfertilized eggs of sea-urchins, as I was able to show this year, live in sterile sea water for five days, or possibly longer, while they die much earlier in ordinary sea water (about two days). The very fact that the eggs of sea-urchins are found mature in the ovary indicates that they are able to live a considerable time after maturation and that they differ in this respect from the starfish egg.

It is, however, a fact that in the same sea water the fertilized and developing sea-urchin eggs live longer than the unfertilized eggs.

It almost seems as if in certain of the higher animals there are eggs which develop only when they are fertilized immediately after leaving the ovary. Under the direction of Professor C. O. Whitman, Harper has shown that the eggs of pigeons are fertilized the moment they leave the ovary. The sperm lives in a gelatinous mass upon the surface of the ovaries,[3] so that provision is made for the necessary contact between sperm and egg. This also does away with the difficulty which many have found in

[1] Loeb and Lewis, *American Journal of Physiology*, Vol. VI., 1902.

[2] Loeb and Lewis, *American Journal of Physiology*, Vol. VI., 1902.

[3] Spermatozoa are in general much longer lived than eggs, even though great differences exist in this regard in different animals. In the spermatic vesicles of the queen bee spermatozoa are believed to remain more than a year after copulations.

explaining how the spermatazoön finds its way to the egg in animals in which fertilization occurs within the body. Definite directive forces are clearly not necessary, since a portion of the spermatazoa must reach the ovary, through their ciliary motion, by way of the uterus and Fallopian tubes. Experiments similar to those made by Harper upon pigeons must yet be made upon mammals. Yet there seems to be no doubt that the mammalian egg of many species is also fertilized before it reaches the uterus. Cases of extra-uterine pregnancy also point to the possibility that fertilization may occur at the surface of the ovary.

VI. The Prolongation of Life and the Theory of Fertilization.

Our experiments seem to have proved that the mature unfertilized starfish egg dies within a few hours through internal changes but that the process of fertilization saves the life of the egg. This is true, not only of the fertilization of the starfish egg by spermatozoa, but also for the chemical fertilization through hydrogen ions. Mr. Neilson succeeded this year in keeping the parthenogenetic larvæ of starfish alive much longer than has thus far been the case (over thirty days), and Dr. Fischer was able to accomplish the same for the larvæ produced osmotically from unfertilized sea-urchin eggs. It is therefore possible that the chemical or osmotic fertilization of these eggs can give rise to as long-lived larvæ as the fertilization of the egg through sperm.

But how does the spermatozoön or the physical and chemical means substituted for it, save the life of the egg and why does the mature egg die when it is not fertilized by sperm or artificial means? I believe that the answer lies in this, that the fertilizing agencies accelerate metabolic processes in the egg which, before fertilization, went on only slowly. After fertilization by sperm or by the chemical or physical means substituted for it, the egg divides and grows, which it did not do before fertilization occurred. Growth is inconceivable without a preponderance of synthetical over hydrolytical processes. I believe it possible that the determining factor in the chemical forces set in motion within the egg through fertilization consists in this that the synthetical processes in the egg are accelerated. If these processes are not inaugurated

or accelerated the egg dies. The wasting of the body in old age also indicates a decrease in synthetical processes. Whether the second critical period occurring in old age is similar to the critical period of the egg cannot yet be determined. Yet it is not impossible that the question of the prolongation of life at this period should pass over into the question of the possibility of accelerating synthetical processes.

We, therefore, come to the conclusion that fertilization accelerates a series of chemical changes (syntheses?) in the egg which do not occur sufficiently rapidly without spermatic, chemical or osmotic fertilization in the eggs of the majority of animals. But why does the mature egg die when these processes are not accelerated, and why does it remain alive before it maturates? The egg must often exist for years in the immature condition in the ovary. In answer I can only suggest *that the processes underlying maturation are at least in some form of a destructive nature (one might think of autolytic processes) which the egg cannot withstand for an indefinite length of time without dying.* In many eggs the velocity of these destructive (autolytic?) processes may be greater than in others and this may determine the differences in the velocity with which the mature, unfertilized egg dies. It is in harmony with this view that when maturation is prevented, or the mature egg is put under condition which inhibit the process of maturation or the chemical processes underlying it, that the life of the egg is lengthened. Lack of oxygen or the addition of an acid work in this way in the case of starfish eggs; a slight addition of potassium cyanide in the case of starfish and sea-urchin eggs. But since all of these substances injure the eggs indirectly and do not entirely do away with the destructive (autolytic?) processes occurring within the egg, life is not prolonged to the same extent by these means as by fertilization in which case life is prolonged not only through an inhibition of the destructive but also through an acceleration of the synthetical processes.

That the chemical processes which underlie maturation are not identical with those which bring about fertilization seems to be supported by the observation made above, that the same means —the treatment with acid—which causes the mature egg to

develop and live beyond the bipinnarian stage, inhibits the maturation of the immature egg. When the *mature* unfertilized eggs of a starfish are introduced for fifteen to sixty minutes into a mixture of 100 c.c. sea water plus 3 c.c. *n*/10 HCl ninety per cent of the eggs can, under favorable conditions, develop into larvæ. If, however, the eggs are introduced into such a solution for the same length of time *before maturation*, the maturation of the eggs is prevented either permanently or for a long time.[1] The difference is still more striking when the eggs are kept for a shorter time in a mixture of 100 c.c. seawater and 5 c.c. *n*/10 HCl. This shows that acid affects the process of development and the process of maturation in opposite or at least different ways.

We must now raise the question, How does the behavior of naturally parthenogenetic eggs, such as the eggs of bees harmonize with these ideas?

In naturally parthenogenetic eggs it seems as if the processes which underlie maturation pass over into those underlying development. But it is possible that this is only apparently the case, and that in reality it so happens that in the processes underlying maturation a metabolic product is formed in the parthenogenetic animals which favors the processes of development. We know that an exceedingly small amount of hydrogen ions suffices to bring about development in unfertilized starfish eggs; that an exceedingly small amount of calcium causes the unfertilized eggs of *Amphitrite* to develop; and that a trace of potassium ions brings about the development of unfertilized *Chætopterus* eggs.[2] It is entirely possible that the specific ions or other substances necessary to start the development of the eggs of the bee are formed within the egg itself through the chemical changes taking place during or after maturation, and that without the formation of these substances development is impossible. In the case of sea-urchins and starfish eggs one might also believe that the processes of maturation and the processes of development pass over into each other. For it has often been observed that the

[1] The eggs which finally maturate in spite of the previous treatment with acid often begin to cleave when maturation is complete and develop into larvæ, while the control eggs kept in normal sea water do not develop.

[2] Loeb, Fischer and Neilson, *l. c.*

unfertilized eggs of these forms after having resided in "normal" sea water for about twenty-four hours begin to cleave shortly before death. This cleavage, however, never goes beyond the two- or four-celled stage. This might be explained by the fact that the eggs begin to die at this time. After I had found this year that the eggs of sea-urchins can still be fertilized after a residence of five days in sterilized sea water (at summer temperature), I decided to study this question of spontaneous cleavage somewhat more closely. If it were true that individual sea-urchin eggs begin to cleave in ordinary sea water after about twenty hours, and cease to develop any further only because they soon die, it would be expected that many or all should cleave when kept alive four or five days, and that a number of them should reach a fairly advanced stage of development. A lot of sea-urchin eggs were distributed into a series of flasks containing sterile sea water. One of the flasks was opened every morning and a careful search was made for developed eggs.

In the course of five days I never found a single divided egg, either in the two-celled stage or in later stages of development. It is possible that during the last days of the experiment a few eggs divided, and that the cleavage cells fell apart. Lewis and I found last year that when eggs are fertilized forty-eight or more hours after their removal from the ovaries they form no membrane and the cleavage cells fall apart. I have corroborated this fact this year. Usually more than one embryo develops from such an egg, because the cells drop apart. I kept this fact in mind and will not disavow that a few small eggs were present, which perhaps represented only half the mass of an ordinary egg. But nearly all the eggs were of normal size, and since small eggs are occasionally found even under normal conditions, the experiment shows that in sea-urchin eggs also the processes of maturation are not continuous with those of cleavage, and that entirely different conditions which we can bring about through the abstraction of water or the entrance of a spermatozoön are necessary that division may occur. It cannot be urged that the sterilized water perhaps prevented the cleavage. When at the conclusion of the experiment these same eggs were fertilized in sterilized water by adding a drop of sperm, they developed to the pluteus stage in

sterile sea water. I, therefore, consider it possible that where authors describe a cleavage of the unfertilized sea-urchin eggs in "normal" sea water, that the sea water or the eggs had in reality suffered some change which had escaped the notice of the observers. One might think of evaporation and increase in the osmotic pressure of the sea water. A very slight increase in the osmotic pressure of the sea water is sufficient to cause the sea-urchin egg to divide into two cells in the course of twenty hours. One might also think of a change in the sea water brought about by the putrefaction of the dead eggs. Finally it is possible that a substance is perhaps formed (for example, an acid) in the dying eggs which brings about a single cleavage.

The relations which exist between maturation and natural death upon the one hand, and fertilization and the prolongation of life upon the other, lead us to the conclusion that a "fertilization" must perhaps come to pass in every egg, even in those naturally parthenogenetic. Only according to our idea, the act of fertilization is not identical with the morphological process which is designated fertilization. It is rather a chemical or a physico-chemical act which accelerates certain (synthetical?) metabolic changes in the egg, which occur in the egg under ordinary conditions also, only much too slowly. The difference between naturally parthenogenetic eggs and the eggs which must be fertilized before they can develop consists perhaps in this, that to the latter the catalytically working substance or complexus of conditions must be added from the outside in order to accelerate the synthetical (?) processes, while in the naturally parthenogenetic eggs these substances are formed within the eggs (possibly in conjunction with the processes of maturation).

The connection between the prolongation of life and fertilization clearly points out that every purely morphological theory of fertilization is incomplete and that a correct theory of this process must have a physco-chemical basis. The means of reaching this basis I see in further attempts at causing development of unfertilized eggs through unequivocal physical and chemical means.

VII. Conclusions.

1. Our observations and experiments seem to show that in the same sea water and under otherwise identical conditions, *mature but unfertilized* starfish eggs soon die, while immature as well as mature but *fertilized* eggs live longer.

2. It seems certain that the rapid death of the mature unfertilized starfish eggs is determined by internal conditions connected with maturation and not by the bacteria contained in the sea water. The proofs for this are: First, mature eggs die just as rapidly in sterilized sea water free from bacteria as in unsterilized water, and secondly, when maturation is prevented artificially the eggs may continue to live in water containing many bacteria.

3. We have shown that oxygen and free hydroxyl ions accelerate the maturation of starfish eggs; that lack of oxygen and a neutral or faintly acid reaction of the sea water inhibit or prevent maturation. The fact that the eggs which remain immature in the ovaries of the starfish maturate when brought into sea water seems to find its explanation in part at least through this.

4. When the maturation of starfish eggs is prevented artificially through lack of oxygen or the addition of an acid to the sea water they remain alive much longer than when they maturate. The eggs in which maturation has already begun or has just been completed seem also to be saved from rapid death by these means.

5. It seems to follow from these facts that the same chemical processes do not necessarily underlie the process of maturation and the process of fertilization. Fertilization by spermatozoa, chemical or physical agencies lengthens the life of the egg, while the changes following the maturation of the egg lead, sooner or later, to death (through autolysis?). It is in harmony with what has been said that the same treatment with acid which brings about artificial parthenogenesis in mature starfish eggs inhibits the process of maturation when used upon immature starfish eggs.

7. These facts corroborate a suggestion which I have made before, that the fertilizing action of the spermatozoön consists in this, that it carries into the eggs substances which accelerate the course of certain (synthetical?) processes in the egg. Such an acceleration might, for example, be brought about through certain ions (for example, the hydrogen ions of nucleic acid), yet the

possibility that such catalytic effects might also be brought about through enzymes or other substances is of course not shut out. Yet this fact must be considered: that we have been able to produce artificially *normal* embryos *capable of development* through ions, while the careful experiments of Gies conducted in my laboratory in which he attempted to find the same to hold for enzymes have thus far failed.

In conclusion I wish to thank my assistant, Mr. Neilson, for the assistance which he rendered me in these experiments.

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The November number of the Biological Bulletin completes the third volume of the series, or the first under the new organization. The number of subscribers has increased from 70 to nearly 200, in addition to thirty copies taken by dealers abroad (in Europe). Six numbers have been issued in seven months, none being issued in September owing to lack of material. Papers received have been published as a rule in from four to eight weeks after receipt. The editors have thus redeemed the promise made in their announcement.

The fourth volume will be started in December, and will be sent to all present subscribers, unless word to the contrary is received. An effort is now being made to extend the circulation abroad. All contributors and subscribers to the Bulletin are urged to use their influence to extend its circulation in this country.

FRANK R. LILLIE,
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